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THE SURVIVAL OF HUMAN ENTERIC VIRUSES IN HOLDING PONDS.(U)
JAN 78 B P SAGIK, S W FUNDERBURG, B E MOORE DAMD17-75-C-5062

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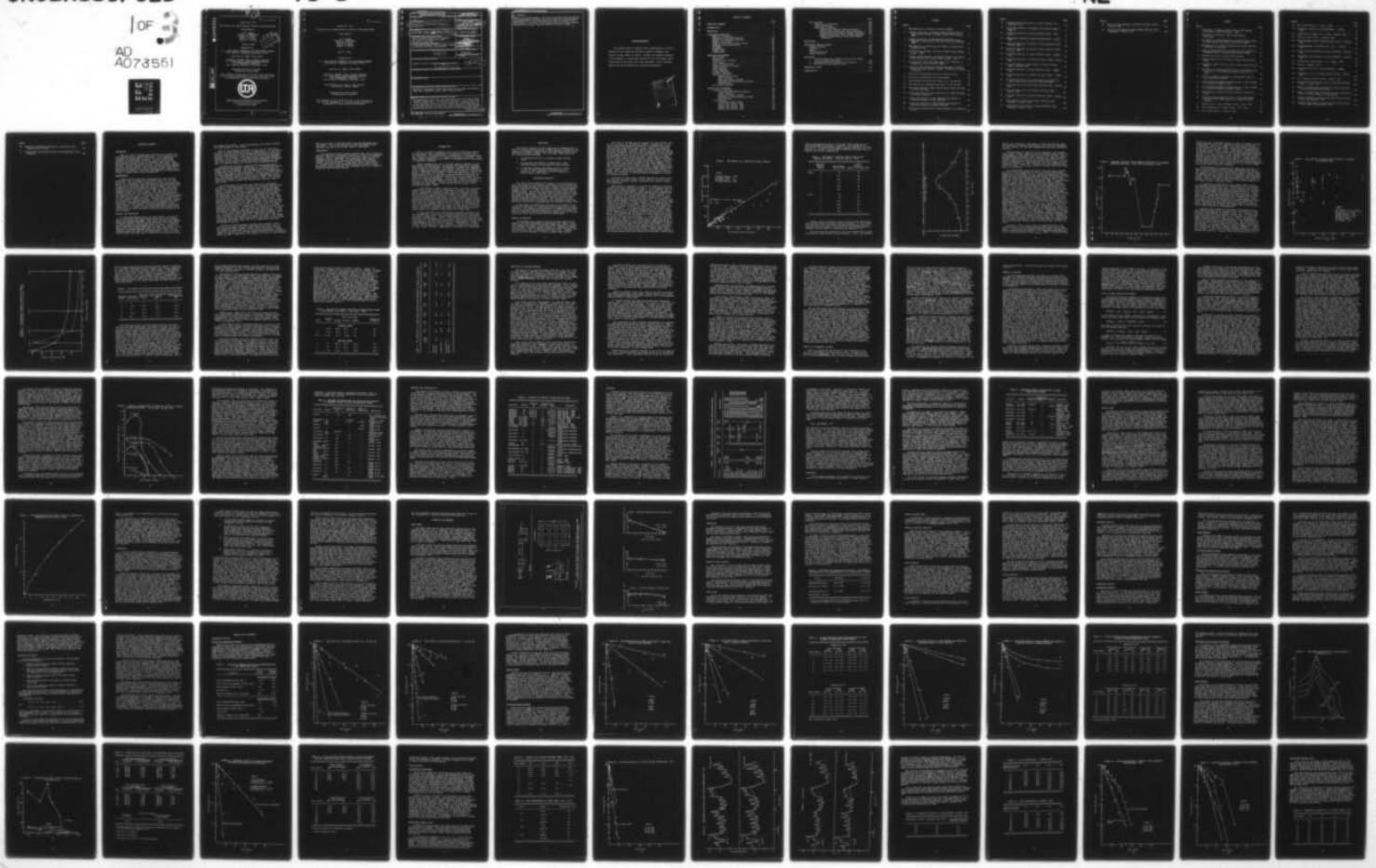

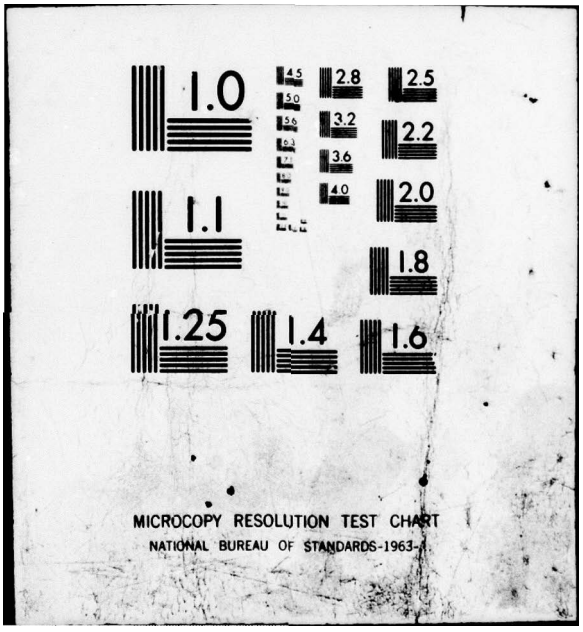
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Report No. 78-1

The Survival of Human Enteric Viruses in Holding Ponds

Final Report

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Steven W. Funderburg
Barbara E. Moore
Ram C. Tripathi
Charles A. Sorber

January 1978

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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The inactivation of Poliovirus I and Coxsackievirus B-3 was studied in model wastewater holding ponds. The ponds were constructed of cast concrete tanks 5 feet in diameter and 18, 30, 42 and 90 inches deep. Experiments were conducted using either settled wastewater (primary effluent) or wastewater that had undergone biological treatment and chlorine disinfection (final effluent). Results indicated that viral inactivation was more rapid during the warmer

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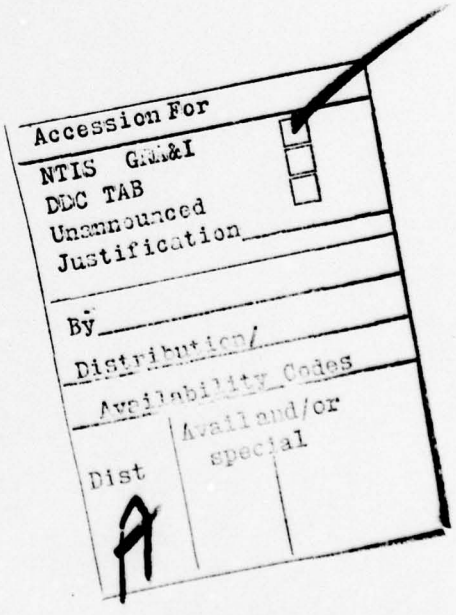
20. Abstract

seasons, and in final effluent. The rate of viral inactivation was enhanced by the development of alkaline pH in holding ponds due to algal metabolism. Approximately 10% of the viruses added to the ponds was detected in the settled solids. The virions in the sediment portion of the ponds underwent inactivation less rapidly than those viruses in the water columns.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Frank R. Hulsey for his technical and moral support throughout the course of this study, to Alan L. Barnett for chemical analysis of the samples, to Elton Ray Collins for his invaluable assistance both in the field and in the laboratory, and to Dorothy Baskin and Helen Ramirez for typing the manuscript.



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BACKGROUND

The U.S. Army is using spray irrigation as a method for land application of treated wastewater. At irrigation sites, a series of holding ponds often is one of the wastewater treatment unit processes included. As such, these ponds serve as the final point in the treatment train where the inactivation of human enteric viruses can take place. Unfortunately, specific survival data for viruses in ponds designed for this use are lacking. The objective of this study was to determine the effects of various holding periods and several depths of wastewater on the long term survival of human enteric viruses in ponds under natural conditions.

EXPERIMENTAL

Model holding ponds were constructed of cast concrete tanks five feet in diameter with depths of 18, 30, 42, and 90 inches. The ponds were situated on a field site in Austin, Texas. The wastewaters used were primary (raw wastewater after 30 minutes of settling time) and final effluents (biologically treated and chlorinated domestic sewage). Field tests were conducted during the months of the year best reflecting the changing seasons in central Texas. The day before the initiation of each test, the ponds were filled with wastewater. The following day, virus (Poliovirus I and Coxsackievirus B-3) was added to each pond at a final concentration of approximately 1×10^4 plaque forming units/ml. The ponds were mixed for 15 minutes using an industrial mixer. Liquid and sediment samples were taken on a regular schedule until no residual virus was recovered. Chemical analyses also were performed on each liquid sample. In addition, selected laboratory studies were conducted in an attempt to more accurately define the factors contributing to viral survival or inactivation observed in the field studies.

RESULTS AND DISCUSSION

In both wastewaters and at all pond depths, the rate of viral inactivation was less rapid during the cooler seasons. During the Spring and Summer, when final effluent pond temperatures were greater than 20C in the afternoon, more than 99.9% of seeded virus was inactivated within 10 days. In contrast, Winter conditions (pond temperatures around 10C) resulted in only a 50% viral inactivation over the same time period. Virus survival in primary effluent ponds was longer than in final effluent ponds, at least in the warm months. From 10 to 40% inactivation took place over a 10-day period during the spring

and summer test series. Viral inactivation was similar between the two effluents in the Winter.

Much of the seasonal difference in the rate of viral inactivation in ponds probably was due to temperature. In laboratory studies, both poliovirus and Coxsackievirus underwent more than 90% inactivation within 5 days at 30C in both final and primary effluent. In contrast, at 4C nearly 99% of the virus remained infectious after 5 days. Other laboratory experiments determined that the rate of viral inactivation could be enhanced by light and, to a greater extent, by the pH of the suspending medium.

In controlled pH tests, stability of both Coxsackievirus and poliovirus was greatest around pH 7.0. Stability fell off on either side of neutrality. The hydrogen ion concentration of primary and final effluent was also increased in the laboratory by culturing algae in the wastewaters. The use of carbon dioxide by these organisms elevated the pH of the wastewaters to over 9.0. Ninety-nine percent of poliovirus in algae-seeded primary and final effluent was inactivated within 10 days. In controls (wastewater without algae) 99% inactivation took more than 30 days at 20C.

The development of an algal population and a subsequent elevation of wastewater pH took place in the model holding ponds. In general, this development occurred faster during the warm months. During the Winter, 1977 field test, the relationship among the growth of an algal community, pond pH change, and the increased rate of viral inactivation was especially striking. The shallower final effluent ponds showed about the same rate of inactivation during the first portion of the test, all three losing 80% of the original virus inoculum within 25 days. The deep pond departed from this scheme, as over 40% of the virions were still recoverable at 27 days. Changes in the initial rates of inactivation were seen in all four ponds. Once the inactivation rate increased, it appeared to be similar in all ponds, including the deep one. In all ponds, an elevation in pH closely followed this change in the rate of viral inactivation. In all four ponds, the pH was uniformly low, initially between 7.2 and 7.5. At the point where the inactivation rate increased, there was an increase in pH to about 8.0. The elevation of pH, in turn, was due to the presence of algae in the ponds. The number of virions decreased fairly rapidly from the ponds' water columns during this and other tests due to pH, temperature and other environmental factors.

Analysis of pond sediments revealed that virions in this portion of the ponds were much more stable than were those in the overlying water columns. After virus was added to a pond the number of infectious units in the water decreased, but the number of virions in the settled solids increased over a period of about one week. Thereafter, the quantity of virus in the sediments

decreased slowly or remained fairly constant depending upon the season. The total quantity of virus appearing in the sediments was about 10% of that added to the ponds.

CONCLUSIONS

The results of these studies indicate that use of multiple ponds is important for maximal reduction of polio and Coxsackieviruses. This is especially so if the first pond is treating wastewater with a high BOD. The remaining ponds enhance effluent quality and encourage algal growth, both of which promote viral inactivation. Pond depth should range from 1.5 to 3.5 feet with a total detention time of at least 30 days. To prevent solids carryover, the discharge point for irrigation should be near the pond surface.

INTRODUCTION

The U. S. Army is presently using several different methods of land application of wastewater at installations within the United States. Land application of wastewater is being emphasized as a practical alternative to advanced wastewater treatment. Further, recent legislation will result in expanded use of this method of wastewater treatment.

Spray irrigation is one of the most popular methods of land application of wastewaters. The design and operational problems associated with this method of wastewater treatment are dependent upon a number of variables. The general topic has been covered in several comprehensive discussions (Corps of Engineers, 1972; Sorber et al., 1972; EPA, 1973; NASULC, 1973; Pound and Crites, 1973). A major research need in the area of spray irrigation has been the determination of the potential human risk of viral disease as a result of spraying wastewater. It has been reported that the probability of inhaling pathogenic aerosols near a spray irrigation site may be significant. Results of recent field studies serve to validate the importance of this problem (Sorber et al., 1976; Katzenelson et al., 1976).

In the design of spray irrigation sites, a series of holding ponds often is one of the unit processes included. Such ponds, unlike conventional oxidation ponds, have a continuous influent, while withdrawal is limited to the growing or irrigation season. The latter is a regional variable. Thus, these ponds serve as holding impoundments accumulating in volume throughout the winter months and being drawn down almost entirely in the summer months. Detention periods are variable, but obviously can range from that time related to maximal pond capacity to almost no detention near the end of the spraying season. In his review of the effectiveness of wastewater treatment processes for the removal of viruses, Sproul (1974) has indicated that there are insufficient data on virus removal by oxidation ponds under field conditions.

It has been shown that conventional secondary treatment cannot be expected to reduce virus concentrations by much more than one order of magnitude. This includes disinfection as practiced currently (Sorber et al., 1974). The holding pond is the next and final point in the treatment chain where viruses can be inactivated. Unfortunately, specific survival data on human enteric viruses in ponds designed for this use are lacking. Consequently, a need exists for information on the effects on viruses in these ponds of time, depth, sunlight, temperature and antagonistic organisms.

OBJECTIVES

The overall objective of this study was to determine the effects of various holding periods, several depths of wastewater and natural environmental conditions on the long-term survival of human enteric viruses in ponds. The individual tasks associated with this objective included:

1. to determine survival of viruses in model holding ponds;
2. to determine the effects of temperature, light, dissolved oxygen, pH and algae on virus survival;
3. to evaluate holding pond design factors in light of the available published literature and the results of the laboratory and field studies conducted under 1 and 2 above.

LITERATURE EVALUATION

The use of waste stabilization ponds in the United States to treat both domestic and industrial wastewaters has increased dramatically during the past few decades. Barsom (1973) has estimated that there were 45 waste stabilization ponds treating domestic sewage in the U. S. in 1945, but that by 1960 the number had increased to 4500. As the only requirements besides proper design are a sufficient amount of suitable land for the ponds and a minimal amount of maintenance, stabilization ponds have great appeal, especially to small communities where monetary resources and trained personnel often are limited. The treatment of organic wastes is accomplished by microbial action, and given sufficient detention time enteric microorganisms are destroyed (Gloyna, 1971).

A great deal of research has been done in an attempt to determine the efficiency of operating stabilization ponds in waste treatment. The purpose of this review is to summarize the literature on waste stabilization ponds and to use this in conjunction with our research to develop preliminary design criteria for optimizing the inactivation of poliovirus in these ponds.

REMOVAL OF ORGANICS

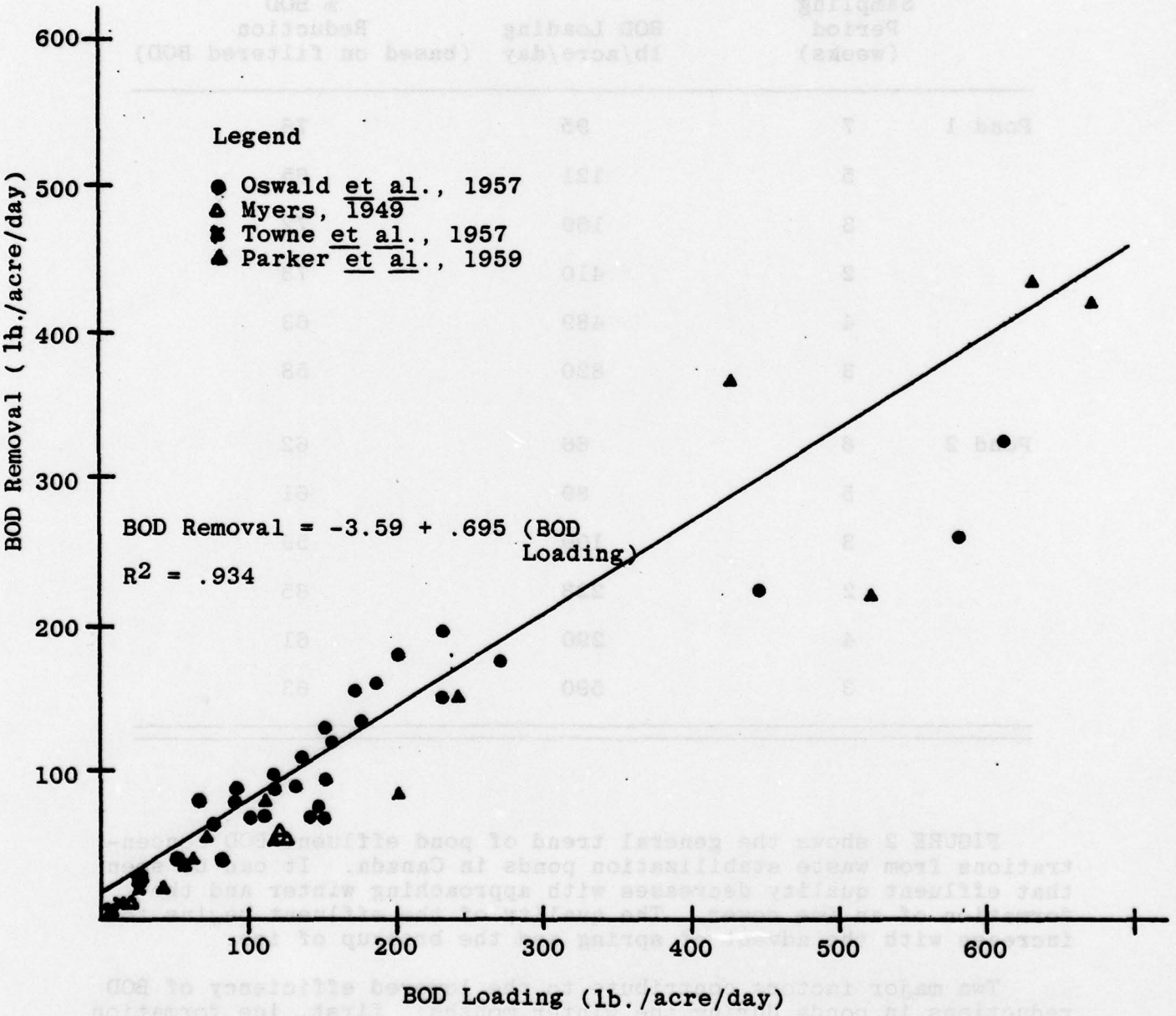
The parameter most commonly studied in waste stabilization ponds is the 5-day biochemical oxygen demand test (BOD_5). This test measures the amount of dissolved oxygen required to biologically degrade the organic matter present in a wastewater sample (Gloyna, 1971). BOD is commonly measured in terms of areal loading (pounds/acre/day) or concentration (mg/liter). Effluent quality is often expressed in terms of percent BOD_5 removal.

The value of the BOD test as a measure of pond effluent quality is the subject of considerable debate. Because much of the organic matter decomposed by bacteria is transformed into algal cell material which itself exerts a BOD, there is a conversion from one form of BOD to another, either of which must be met by the receiving stream. However, some workers feel that the BOD exerted on a stream by algal cells is not comparable to that exerted by raw sewage because, in the latter case, nearly all of the oxygen demand would be confined to a small volume near the sewage outfall, while algae, being planktonic, would exert their demand over a relatively large area (Stumm and Morgan, 1962). The result of this debate is that some workers filter their samples before reporting BOD, some report the BOD for unfiltered samples, while many do not indicate which method was used. The confusion is further complicated by the fact that filtering a sample removes particulates other than algae which may exert an oxygen demand (Neel et al., 1961). Nevertheless, removal of BOD is still the most widely used criterion for measuring waste stabilization pond efficiency and, therefore, is the most important parameter in nearly all predictive models.

According to Young (1974), McGarty and Pescond (1970) state that the most important factors in the reduction of BOD within a stabilization pond are loading, detention time, number of ponds, and temperature.

BOD removal as reported in a variety of papers is plotted as a function of areal loading in FIGURE 1. It is apparent that BOD removal from waste stabilization ponds is fairly constant over a wide range of loading regimes. Parker et al., 1959, studying an anaerobic lagoon receiving loadings from 630-1883 lb BOD/acre/day found little variation in the percentage of waste removed. In similar fashion, in a facultative pond in series with the above lagoon, loadings from 54-191 lb/acre/day of BOD did not significantly reduce removal efficiencies below 95%. In a study of ponds receiving raw sewage at Fayette, Missouri, Neel et al. (1961) also found little variation in organic removal as BOD loadings were increased from 20-1000 lb/acre/day. Merz et al. (1957) observing two ponds in series at Mojave, California, found the results tabulated in TABLE 1. It is apparent here, too, that loading rate differences of up to an order of magnitude did not alter significantly the ability of the ponds to remove organic matter. Towne et al. (1957) summarized the results of their study on waste stabilization ponds in five municipalities in the Dakotas by stating, "in spite of differences in loading . . . there were no striking differences in (BOD) reduction among the five stabilization ponds during the four seasons." In another Dakota study Olson et al. (1968) determined that loadings from 28-250 lb BOD/acre/day did not effect stabilization pond efficiency during the summer. A survey of 26 stabilization ponds in Iowa (Stouse, 1964) also indicated that effluent quality during the summer months was not altered by loadings of up to 500 lb/acre/day of BOD. Porges (1963) found little correlation between effluent quality and organic loading in five waste stabilization pond systems in California. Parker et al.

FIGURE 1. BOD REMOVAL AS A FUNCTION OF AREAL LOADING.



(1950) investigated the effect of various organic loadings on a facultative pond in Melbourne, Australia. At loadings of 51.7, 65.0 and 66.5 lb BOD/acre/day removal remained between 90 and 100%. However, at loading of 105 and 202 lb/acre/day efficiency was reduced to 76 and 31%, respectively.

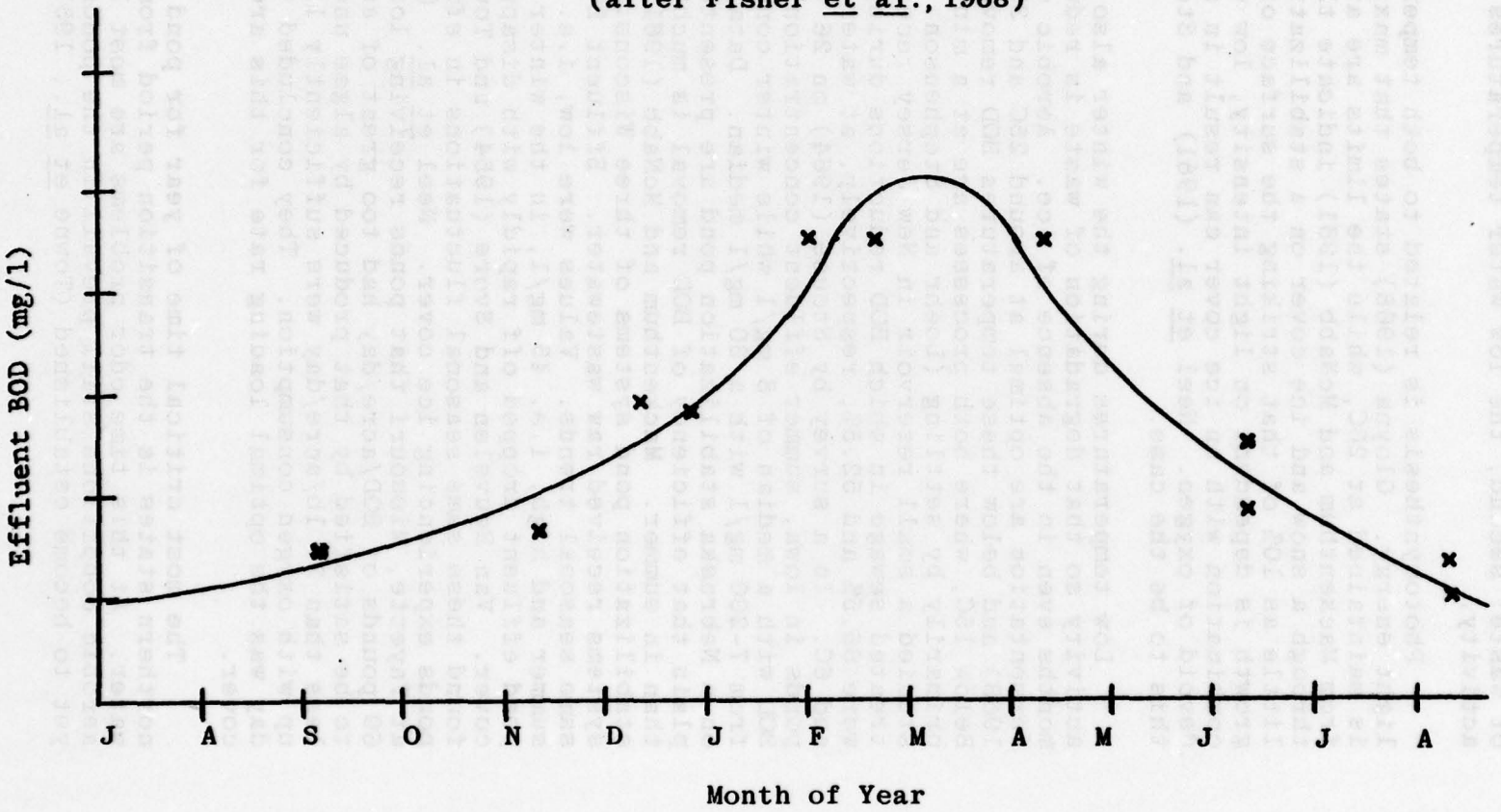
TABLE 1. THE EFFECT OF ORGANIC LOADING RATE ON BOD REMOVAL EFFICIENCY. (from Merz *et al.*, 1957)

	Sampling Period (weeks)	BOD Loading lb/acre/day	% BOD Reduction (based on filtered BOD)
Pond 1	7	95	76
	5	121	65
	3	169	72
	2	410	73
	4	489	63
	3	820	58
Pond 2	6	66	62
	5	89	61
	3	109	59
	2	223	65
	4	290	61
	3	590	63

FIGURE 2 shows the general trend of pond effluent BOD concentrations from waste stabilization ponds in Canada. It can be seen that effluent quality decreases with approaching winter and the formation of an ice cover. The quality of the effluent begins to increase with the advent of spring and the breakup of ice.

Two major factors contribute to the lowered efficiency of BOD reductions in ponds during the winter months: first, ice formation

FIGURE 2. SEASONAL VARIATION OF EFFLUENT BOD CONCENTRATIONS OF STABILIZATION PONDS IN CANADA. (after Fisher et al., 1968)



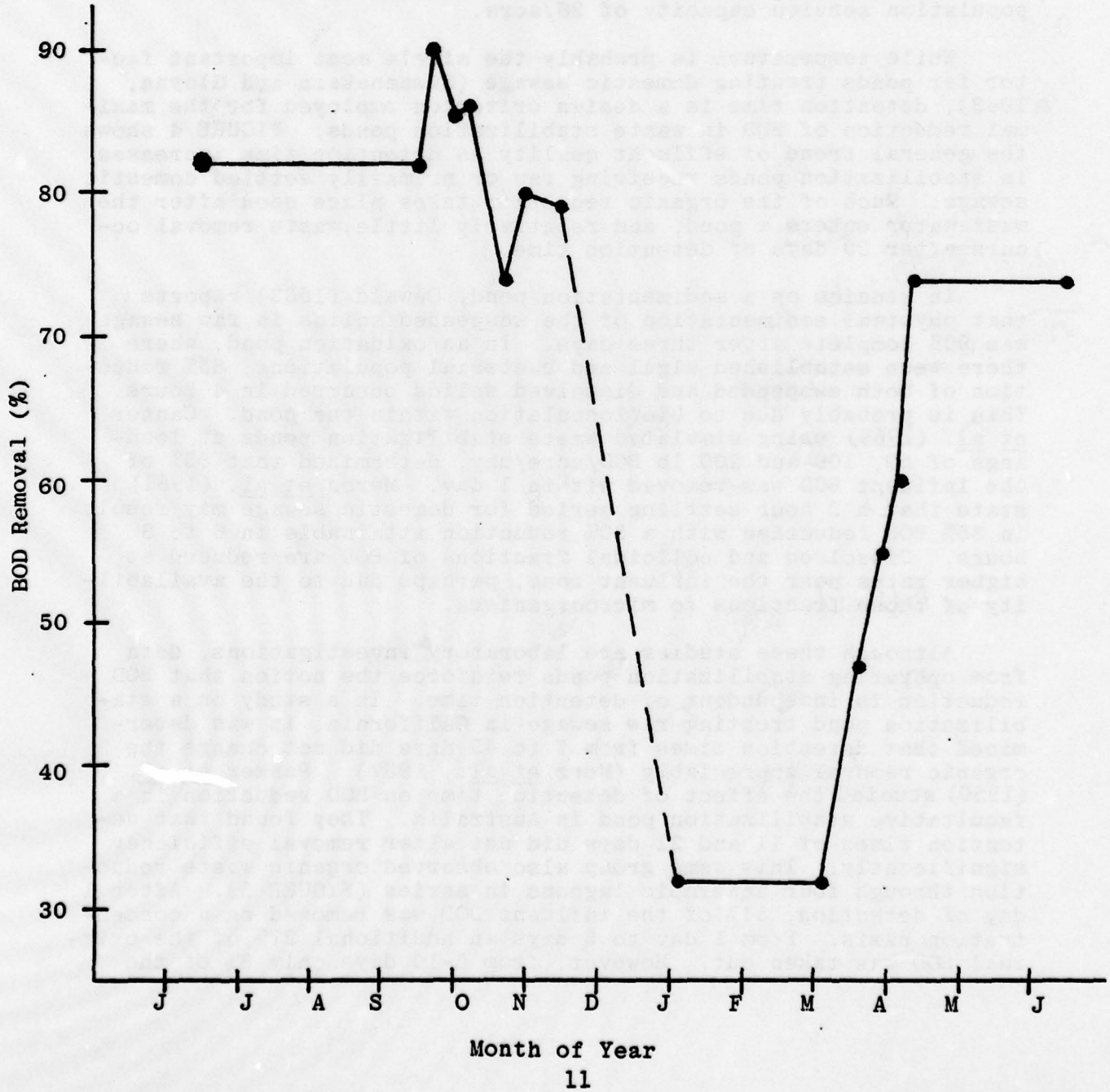
results in a reduction in the amount of light entering the pond, thus reducing or halting photosynthesis and oxidative degradation of waste; second, the low water temperatures slow down biological activity.

Photosynthesis is related to both temperature and incident light energy. Gloyna (1968) states that maximum oxygen production is maintained at 20C, while the limits are at 4C and 35C. Data from Mackenthum and McNabb (1961) indicate that light transmitted through a snow and ice cover on a stabilization pond can be as little as 10% of that striking the surface of the cover. As algal growth is dependent on light intensity, low water temperatures in combination with an ice cover can result in a stabilization pond devoid of oxygen. Neel et al. (1961) and Stouse (1964) have shown this to be the case.

Low temperatures during the winter also slow down biological activity so that degradation of waste is reduced during these months even in the absence of ice. Aerobic oxidation and methane fermentation are optimal at around 25C and 35C respectively (Oswald, 1968) and below these temperatures BOD removal is slowed down. Below 15C, where both processes are at a minimum, BOD is removed primarily by settling (Loehr and Stephenson, 1965). Ridenour (1933) studied a small reservoir in New Jersey receiving secondarily treated sewage in which BOD reductions during summer and winter were 66.5% and 52.5%, respectively, at water temperatures of 21C and 6C. In a survey by Stouse (1964) on 26 waste stabilization ponds in Iowa, summer effluent concentration ranged from 4-16 mg/l BOD with a median of 8 mg/l while winter concentrations ranged from 7-200 mg/l with a 30 mg/l median. Data from an investigation on a Nebraska stabilization pond are presented in FIGURE 3. It is plain that efficiency of BOD removal is much lower during winter than in summer. Mackenthum and McNabb (1961) compared the waste stabilization pond systems of three Wisconsin towns in which all systems received raw wastewater. Effluent BOD values showed the same seasonal trends. Values were low, i.e. 10-15 mg/l during the summer and high, i.e. 45 mg/l, in the winter months. BOD of the pond effluent dropped off rapidly with disappearance of the ice cover. Van Heuvelen and Svore (1954) and Towne et al. (1957) also found these same seasonal fluctuations in effluent BOD in Dakota ponds experiencing ice cover. Neel et al. (1961) noted in a study at Fayette, Missouri that ponds receiving loadings of greater than 60 pounds of BOD/acre/day had too great of an oxygen consumption to be satisfied by that produced by algae under ice. Loadings of less than 20 lb/acre/day were sufficiently light for algae to keep up with oxygen consumption. They concluded that 40 lbs BOD/acre/day was the optimal loading rate for this area due to winter ice cover.

The most critical time of year for pond operation in the northern states is the transition period from ice cover to open water. At this time odor problems are most likely to occur as anaerobic conditions still prevail in the ponds and aerobiosis has yet to become established (Towne et al., 1957). To avoid the odor

FIGURE 3. SEASONAL VARIATION IN BOD REMOVAL EFFICIENCY IN A NEBRASKA STABILIZATION POND. (after Neel and Hopkins, 1956)



problem and yet to keep BOD removals as high as possible, northern states generally have used loadings of around 20 lb/acre/day (Suwannakarn, 1963; Svore, 1968). This has resulted in reduction of BOD concentrations from 74-98% during open water seasons and 70-96% under ice (Van Eck, 1959). Canter et al (1969) surveyed the design criteria used by waste stabilization pond operations in the United States. States above 42° latitude experience prolonged periods of ice cover and have a mean loading rate of 26 lb BOD/acre/day or an average population of 124/acre. States between 37° and 42° latitude have a mean loading rate of 33 lb/acre/day or average population service capacity of 189/acre, as these states experience short periods of ice. States below 37° latitude experience no ice cover and have a mean loading rate of 44 lb or an average population service capacity of 26/acre.

While temperature is probably the single most important factor for ponds treating domestic sewage (Suwannakarn and Gloyna, 1963), detention time is a design criterion employed for the maximal reduction of BOD in waste stabilization ponds. FIGURE 4 shows the general trend of effluent quality as detention time increases in stabilization ponds receiving raw or primarily settled domestic sewage. Much of the organic reduction takes place soon after the wastewater enters a pond, and relatively little waste removal occurs after 20 days of detention time.

In studies on a sedimentation pond, Oswald (1963) reports that physical sedimentation of the suspended solids in raw sewage was 90% complete after three days. In an oxidation pond, where there were established algal and bacterial populations, 85% reduction of both suspended and dissolved solids occurred in 4 hours. This is probably due to bioflocculation within the pond. Canter et al. (1969) using simulated waste stabilization ponds at loadings of 50, 100 and 200 lb BOD/acre/day, determined that 85% of the influent BOD was removed within 1 day. Meron et al. (1961) state that a 2 hour settling period for domestic sewage may result in 35% BOD reduction with a 50% reduction attainable in 6 to 8 hours. Dissolved and colloidal fractions of BOD are reduced at higher rates near the influent zone, perhaps due to the availability of those fractions to microorganisms.

Although these studies are laboratory investigations, data from operating stabilization ponds reinforce the notion that BOD reduction is independent of detention time. In a study on a stabilization pond treating raw sewage in California, it was determined that detention times from 7 to 43 days did not change the organic removal appreciably (Merz et al., 1957). Parker et al. (1950) studied the effect of detention time on BOD reduction in a facultative stabilization pond in Australia. They found that detention times of 11 and 21 days did not alter removal efficiency significantly. This same group also observed organic waste reduction through four anaerobic lagoons in series (FIGURE 5). After 1 day of detention, 54% of the influent BOD was removed on a concentration basis. From 1 day to 5 days an additional 27% of the original BOD was taken out. However, from 5-10 days only 8% of the

FIGURE 4. BOD REDUCTION IN STABILIZATION PONDS AS A FUNCTION OF DETENTION TIME.

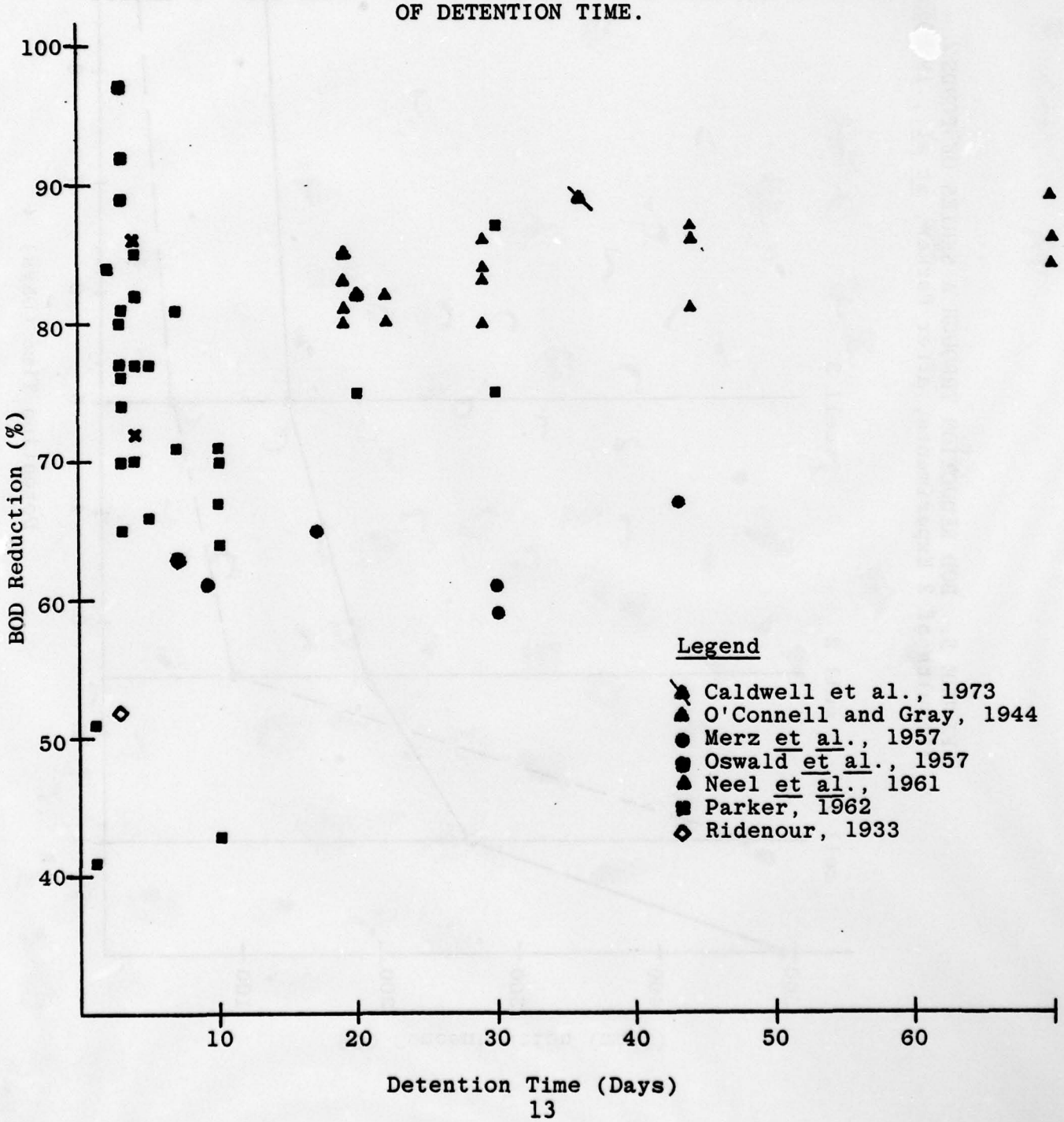
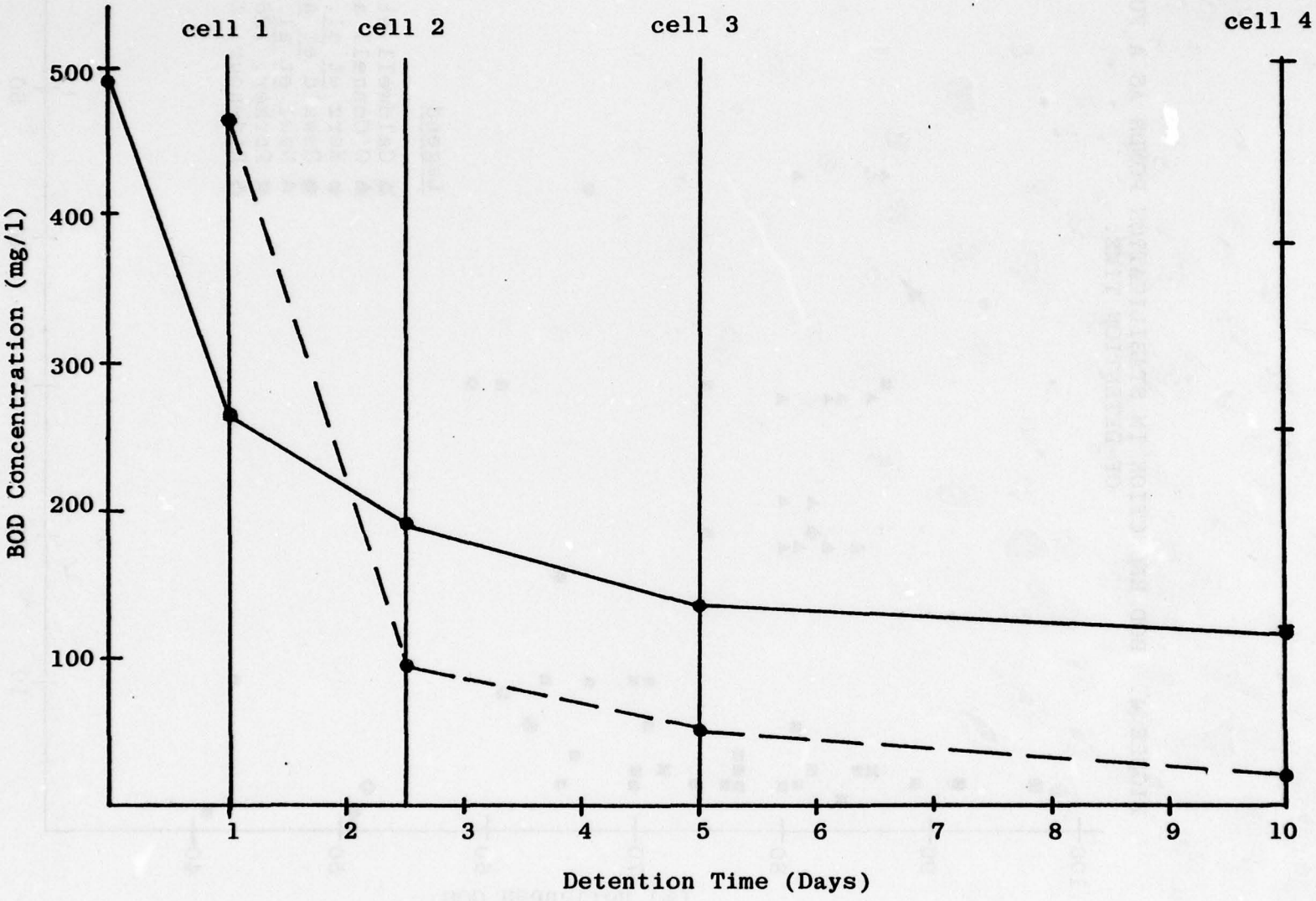


FIGURE 5. BOD REDUCTION THROUGH A SERIES OF PONDS.
(Results of 2 Experiments, after Parker, et al., 1950)



organic waste originally entering the pond was removed. Parker et al. concluded that detention time beyond 5 days was not desirable to reduce BOD in anaerobic lagoons. Meron et al. (1965) sampled two waste stabilization ponds connected in series at various distances from the inlet. Their results are presented in TABLE 2. Again it is apparent that the majority of BOD is removed before 10 days of detention. Neel et al. (1961) Towne et al. (1957) and Mackenthum and McNabb (1961) also found that varying detention time within ponds does not have significant effect on their ability to reduce BOD levels.

TABLE 2. THE EFFECT OF DETENTION TIME ON BOD REMOVAL EFFICIENCY

Sampling Point	Detention Time (Days)	Total BOD Reduction (%)	Reduction Between Sampling Point % of Initial BOD	% of Remaining BOD
1	0	0	0	0
2	6.5	72.5	72.5	72.5
3	13	81.2	8.7	31.0
4	19.5	85.0	3.8	18.5
5	26	87.4	2.4	16.0

The observation that relatively short detention times are sufficient for BOD reductions of above 80% is curious in light of the fact that most pond designs are based on organic removal and frequently employ detention times as long as 60-120 days (Van Heuvelen et al., 1960). Oswald and Gotaas (1957) state that the reason a minimum detention period is necessary is that the oxygen required to decompose the influent waste may be fixed by light and temperature, but they suggest that not less than 1 day during the summer nor more than 6 days during the winter is sufficient for this process at latitudes below 40°. In pilot plant studies in California (37° latitude) Oswald et al. (1957) found that detention times of 3.5 days during the summer were sufficient for greater than 80% reduction of BOD loadings in the range of 225-250 lb/acre/day. They recommended 5 days detention and loadings of 100-125 lb/acre/day in the winter at this same latitude. Hermann and Gloyna (1958) suggest that 3.5 days detention is sufficient for 85-90% removal of BOD with an influent level of 200 mg/l at 35C. This latter figure was revised by Gloyna (1968) to 7 days under the same conditions. In view of these calculations, the 117

day average detention time employed in states above 42° latitude, 82 days between 37° and 42°, and 31 days below 37° (Canter et al., 1969) are probably too long if reduction of BOD is the major factor under consideration.

The depth of a pond is closely related to detention time and may be involved in the reduction of BOD. Unfortunately, as changes in depth usually result in changes in detention time, few studies have been conducted on the effects of depth on BOD in stabilization ponds. Williford and Middlebrooks (1967) studied two stabilization ponds in Mississippi operating in parallel and receiving raw wastewater. One pond was 5.0 feet deep and loaded at 20 lb BOD/acre/day. The second pond was 3.0 feet deep and loaded at 33 lb/acre/day. The average effluent concentration differences of 45 and 55 mg/l, respectively, were attributed to the different loading rates and not to the variations in depth. Parker et al. (1950) could find no significant differences in effluent quality in a pond that was operated at 1.5 and 3.0 feet. Mills (1961), operating a .0022 acre experimental pond in Florida at loadings from 193-309 lb BOD/acre/day, found reduction efficiencies of 90% at depths of 2.5 and 3.5 feet. Reporting on two otherwise identical ponds in Fayette, Missouri operating at depths of 2.5 and 5.0 feet, Claire et al. (1961) found that loadings of 60 lb/acre/day produced an average BOD concentration in the effluent of 44 and 40 mg/l, respectively. Young (1974) reports studies by Clark et al. (1970) and Purushothaman (1970) in Alaska and India in which depth had no significant effect on BOD reduction, also.

Parker et al. (1950) state that within the range that most waste stabilization ponds are presently operated, changes in depth will probably not affect BOD removal. The minimal liquid depth recommended in the United States is 2.0 feet, the maximal recommended is 6.0 feet, and the average recommended depth is 4.0 feet (Canter and Englande, 1970).

A final design feature considered important in removal of BOD is the use of serial ponds. Multiple ponds effectively increase detention time and, therefore, expose the organic waste to further oxidation or fermentation. However, as has been discussed above, long detention times are of doubtful significance with regard to the reduction of organic matter. For this reason most of the BOD will be removed by the first pond in the series, and the following ponds will serve mainly to "polish" the effluent.

The waste treatment facility at Hancock Air Force Base, New York consisted of two stabilization ponds in series. Pond 1 was 1.7 acres; pond 2, 6.9 acres. In studies by Nemerow and Bryson (1963) it was found that 69% of the BOD was removed in the first cell, while the second further reduced the organics by 17%, despite the fact that the second cell had over three times the surface area that the first did. Parker et al. (1959) reported on a similar situation in Melbourne, Australia. A 34-acre anaerobic pond in series with a 276 acre aerobic pond removed 70% and 30%, respectively, of the applied BOD. The removal of BOD from serial

stabilization ponds in Texas is shown in TABLE 3. Again, the first pond is responsible for over half the total removal, although the effluent quality is considerably enhanced by the rest of the series. It is apparent from TABLE 4 that with eight ponds in series only the first four make any substantial contribution to reduction of BOD. Removal in the first pond (detention time 3.8 days) was 1340 lb/acre/day. Removal from the second through fourth ponds (total detention time 42.8 days) was 32 lb/acre/day, while in ponds 5 through 8, with a total detention time of 123.5 days, the loss of BOD is inconsequential (Parker, 1962). Parker speculates that the decrease in BOD removal between successive ponds may be due, in part, to decreased bacterial activity associated with the loss of organic matter in previous ponds. A number of other studies in Texas (Ulbrich, 1967), California (Porges, 1963; and Merz et al., 1957), Mississippi (Williford and Middlebrooks, 1967), North Dakota (Van Heuvelen and Svore, 1954), Australia (Parker et al., 1950; 1959), Mexico (De la Espino and Aguirre Martinez, 1976), and Israel (Meron et al., 1965) reiterate the fact that the majority of BOD reduction takes place in the first pond in a series, but that the following ponds are able to enhance the effluent quality.

TABLE 3. THE EFFECT OF SERIES OPERATION OF STABILIZATION PONDS ON BOD REMOVAL EFFICIENCY. (from Myers, 1949)

Pond	Surface Area	BOD lb/acre/day Loading	Removal	% Removal	Cumulative % Removal
		Killeen, Texas			
1	4.52	124.0	56.0	45	45
2	4.75	65.0	26.0	40	67
3 & 4	11.46	18.2	10.25	65	88
		Decatur, Texas			
1	1.3	121.0	59.0	48.7	48.7
2	1.5	54.0	35.0	65.0	82.0
3	1.78	16.0	5.3	33.0	88.0

TABLE 4. BOD REDUCTION IN 8 WASTE STABILIZATION PONDS OPERATED IN SERIES IN AUSTRALIA

	Raw Sewage	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Pond 8
Effluent BOD mg/l	521	100	46	22.7	22.7	13.5	10.1	10.0	10.6
% Reduction	--	80.8	54	51	0	41	20	1	6
90 Cumulative Reduction	--	80.8	91.2	95.7	95.7	97.1	98.1	98.1	98.1

REDUCTION OF COLIFORM BACTERIA

The function of a waste treatment facility is not only to reduce the amount of organic matter present in the sewage, but also to insure a significant reduction in pathogenic agents. To measure this end result, microorganisms of the coliform group, especially Escherichia coli, have been used as indicators of the degree that a treated wastewater has been rid of potentially harmful microbes.

It is generally felt that waste stabilization ponds are efficient in destroying most of the influent coliforms. In an evaluation of stabilization pond literature, Fitzgerald and Rolich (1958) state, "in nearly all instances the bacterial counts have been lowered to less than 1% of the original concentration. The E. coli counts have generally been reduced from several hundred thousand to less than 100/ml . . ." A joint report of the U. S. Dept. of Health, Education and Welfare and the Boards of Health of North and South Dakota restated the ability of stabilization ponds to remove 95-99% of the influent coliforms (Van Eck, 1959).

Even though reduction efficiencies, on a percent basis, are very good, an examination of APPENDIX A indicates that the number of organisms discharged from pond systems very often can be as high as 10^5 per 100 ml. As regulations for secondary treatment of wastewater call for no more than 200 fecal coliforms per 100 ml of effluent for 30 consecutive days and no more than 400/100 ml during any consecutive 7 day period (U. S. Environmental Protection Agency, 1973), it is apparent that the ability of stabilization ponds to reduce coliforms is not adequate enough to meet the 1973 regulations. The problem of high coliform counts can be especially troublesome as many ponds discharge into small streams where dilution and continued purification are minimal. Probably one of the major reasons for the high number of coliforms discharged from many stabilization ponds is that most ponds are primarily designed to achieve maximum reduction of BOD. In fact, Young (1974), in a survey of facultative stabilization ponds literature, states that--he was unable to find any predictive designs that modeled pond parameters other than organic removal. Despite this, however, some studies have been performed that have monitored the removal of coliforms from pond systems. As stabilization ponds are complex ecological systems and have no simple effects on the organisms within them, there are a number of parameters, acting alone or together that may affect coliform survival.

Pratt et al. (1944) claimed to have isolated an antibacterial substance, chorellin, from Chlorella, a common algal genus in waste stabilization ponds. Caldwell (1946), after studying coliform reductions in California ponds, also asserted that the removal of these organisms was due to a substance liberated by algae. However, Oswald and Gotaas (1957), Hodgson (1964), and Gameson and Saxon (1967) could find no such anticoliform substance in algae cultures.

An indirect effect that algae may have on the survival of coliforms is the pH change that algae induce in stabilization ponds. As stated above, Caldwell (1946) attributed coliform die-off to algae, but he felt that the pH in the ponds under investigation was not high enough to induce die-off. In India, Parhad and Rao (1963) attributed coliform removal to pH changes within the ponds. In a laboratory investigation, Parhad and Rao (1974) found rapid die-off of E. coli in the presence of various algal species when the pH went above 9.0. If, however, the cultures were buffered to 7.5, survival was fairly constant for 8 days at room temperature. Parhad and Rao attributed much of the rapid removal of coliforms in waste stabilization ponds to the high pH levels attained in these systems. Yousef (1962) also found that alkaline pH in stabilization ponds contributed to coliform reduction.

Algae may possibly reduce the number of coliforms by developing aerobic conditions within ponds. Slanetz et al. (1972) and Neel and Hopkins (1956) found great survival of coliform organisms during periods of anaerobiosis.

It has been known that light has bactericidal properties (Gates, 1929), and thus sunlight may be effective in reducing coliform numbers in stabilization ponds. Two reports in the literature lend credence to this hypothesis. Oswald and Gotaas (1957) found accelerated die-off of coliforms in light-saturated algal cultures as compared to those that were not saturated. Gameson and Saxon (1967) found that the logarithm of coliform count decreased regularly with the increase of cumulative radiation. It was also determined that the surface radiation required to produce 90% mortality increased during the winter and with depth of immersion. The most effective wavelengths in coliform destruction were ultraviolet to visible blue.

Like variations in light intensity and duration, the changing of temperatures and seasons may affect coliform die-off. Many workers have studied the relationship between seasons and removal of coliforms from stabilization ponds. Slanetz et al. (1972) studying a series of pond systems in New Hampshire found that fecal coliform survival was better during the winter months when the water temperatures were below 10C. The median coliform MPN for the effluent of 26 Iowa stabilization ponds in winter was 2.3×10^6/100 ml. During the summer the median was nearly two orders of magnitude lower, 4.5×10^4/100 ml (Stouse, 1964). Klock (1971), investigating Arizona waste stabilization ponds, found that the coliform survival rate constant, K_{10} (based on Chicks Law), increased with increasing temperatures. Studies in Ohio (Geldreich et al., 1964) and Wisconsin (Mackenthum and McNabb, 1961) have also confirmed this trend of greater coliform survival during the winter and, presumably, at lower temperatures.

These results are somewhat contrary to what would be expected to occur if coliform survival were based solely on the prevailing pond temperature. These organisms naturally reside in the gut of

warm-blooded animals where temperatures exceed those found in stabilization ponds. Therefore, increased vulnerability of coliforms in the warm months probably is not due to temperature per se, but to some heat-dependent factors within the ponds. Klock (1971) speculated that when coliforms are expelled from the host they revert to an endogenous metabolism, due either to a lack of available substrate or to competition for it. Thus, over a period of time, substrate exhaustion results with an accompanying die-away of the population. As metabolic rates are lower at reduced temperatures, more coliforms would survive to be discharged with the effluent during the winter than in summer.

Although algae, pH, sunlight, and temperature may contribute to the die-away of coliforms in stabilization ponds, they are largely uncontrolled and as such cannot be considered as major design criteria. However, organic loading, detention time and pond number are three variables that can be controlled in pond design and may have effects on coliform survival.

Claire et al. (1961) Hodgson (1964), Neel et al. (1961) and Canter et al. (1969) found a decrease in the percent removal of coliforms as organic loadings increased. Parker (1962) determined that the percent reduction in coliforms increased as the concentration of BOD decreased. It is not clear from any of these studies, however, whether this is due to a greater competition for substrate with decreased concentrations of BOD or if the increased organic content of the waste may be affording some protection to the microorganisms. In contrast, Franzmanthes (1970), Little et al. (1970) and Stouse (1964) could find little relationship between organic loading and coliform survival.

A number of studies have indicated that detention time in single ponds is related to reduction of total coliform densities in the effluent. Little et al. (1970) examined nine stabilization ponds in the southeastern U. S. and found a direct correlation between detention time and die-off of fecal coliforms. Warrington (1952) found that a pond treating secondary effluent in Texas could reduce coliform MPN from 1.1×10^6 to 4.5×10^2/100 ml with a detention time of 30 days. Neel et al. (1961) Malone and Bailey (1969), and Parker (1962) also found detention time to be important in the reduction of coliform numbers within ponds. Loehr and Stephenson (1965) attributed a 50% removal of coliforms from a maturation pond in Kansas to a detention time of only 1.8-3.3 days. Similarly, a New Jersey maturation pond, with a detention time of 2.8 days, removed only 76% of E. coli during the summer and 40% in winter (Ridenour, 1933).

McKinney et al. (1971) state that "coliform reductions in oxidation ponds are the result of starvation rather than from toxic materials or from unusual predation." Thus, they concluded that coliform die-off is a function of detention time primarily. At 20C there should be a 99% die-off of coliforms in 20 days, 99.99% in 40 days and 99.9999% in 60 days. They recommend the use of multiple ponds operated in series to prevent short circuiting.

Coliform reduction is most efficient when stabilization ponds are operated in series, especially when the ponds number three or more. Slanetz et al. (1972) compared stabilization ponds in New Hampshire for coliform reduction efficiencies. When two ponds were operated in series at 17-26C there was a 95-99% reduction in both total and fecal coliforms. However, when the ponds were operated in a series of three or four ponds, less than two organisms per milliliter were found in the effluent over a temperature range of 10-26C (a 7% reduction). In South Africa, Marais (1974) collected data from four stabilization ponds connected in series. Here, detention time was 2.5 days in each pond (10 days total), and fecal coliforms were reduced by 99.91%. In a single pond with a 10 day detention period, Marais found a 95% reduction. Shindala and Mahlock (1974) observed a similar situation in Mississippi. A three-cell system proved more effective in reducing coliforms over a similar single-cell system. There was a significant reduction in both total and fecal coliforms between consecutive ponds. Franzmanthes (1970) in a literature survey could find no correlations between fecal coliform reduction and either organic loading rates or detention times within single ponds. He did, however, find that pond number appeared to be significant in removing coliforms. Little et al. (1970) Parker et al. (1950) and Parker (1962) also determined that pond number was important in reducing coliform populations to acceptable levels.

Why multiple ponds are more efficient in promoting coliform die-off than are single ponds with similar detention times is not clear when one notes that detention period is the only factor that has been correlated reliably with the removal of coliforms and that temperature has been viewed as being of secondary importance (McKinney et al., 1971). The Missouri Basin Engineering Health council recommends long periods of detention in multiple ponds to reduce the chance of short circuiting and so insure maximum coliform reduction (Van Heuvelen et al., 1960). Joshi et al. (1973) compared a three-cell with a two-cell system for efficiency of bacteria removal in Israel. The fecal coliform removal efficiencies were 99.942% and 93.0%, respectively, despite the fact that the two-pond system had a detention period longer by 5 days. They attributed their results to the greater length of liquid travel in the former and to the fact that algae could communicate between ponds in the three-cell system due to surface overflow connections between ponds. Several workers have shown that algae concentrations increase among the first few ponds of a series (Parker, 1962; Hodgson, 1964). If algae are involved in the reduction of coliforms, then series operation of ponds may enhance this removal by encouraging maximum algal growth.

REMOVAL OF PATHOGENIC BACTERIA

There are relatively few reports in the literature on the survival of pathogenic bacteria, and of these, the majority are concerned with Salmonella. Unfortunately, most of these studies are not sufficiently quantitative to produce generalizations about

the efficiency of stabilization ponds in pathogen reduction. O'Connell and Gray (1944) cite a study in Java in which the concentrations of Salmonella typhi ranged from 1 to 41 organisms per milliliter in raw sewage. Effluent from a stabilization pond was consistently negative for the organism. Two influent samples to a stabilization pond in India were positive for S. typhi, whereas the effluent samples were always negative (Parhad and Rao, 1963). Cody and Tischer (1965) examined 14 samples of effluent from a stabilization pond. Four yielded Salmonella isolates and none revealed Shigella species. The density of the Salmonella was reported at less than 1/ml. Kott (1973), in Israel, reported finding Salmonella in stabilization pond influent, but he could not detect this organism in the effluent. The influent to three oxidation ponds operating in series in India with a total detention time of 7 days had a concentration of 4-540 Salmonella organisms/100 ml. This microorganism was not completely eliminated from the pond effluent (Joshi et al., 1972).

Several investigators have reported the effects of temperature on the survival of Salmonella. Sidio et al. (1961) inoculated S. typhi into stabilization pond influent and found complete destruction of the organisms within 12 days at 20C. Hok (1963) observed that the reduction of these organisms to 10/ml took 22 days at 10C but only 2 days at 20 to 30C. In wastewaters with high organic content destruction may take 3 to 5 days at 20C (Hsu and Kruse, 1967). Slanetz et al. (1972) found survival was prolonged in stabilization ponds during the winter at temperatures below 10C as compared to the summer months when temperatures were 17 to 26C.

Serial operation of ponds appears to enhance the reduction of Salmonella. The first pond in a series of two was able to remove 90.2% of the influent S. typhi. The second brought total reduction in the final effluent to 99.5%. This same paper reported removal of S. typhi from four ponds in series with a total detention of 10 days. Pond 1 removed only 29.3% of the influent organisms. The following three brought the total removal to 86.2% (Coetzee and Fourie, 1965). Slanetz et al. (1972) were able to isolate Salmonella from the majority of pond effluent samples throughout the year. However, they did find that three or four ponds in series were able to effect a greater reduction in these organisms than did single ponds or two ponds in series. Despite the fact that two cells in series had a lighter organic loading and a longer detention time than did a three-cell system, the latter was more efficient in Salmonella reduction. Joshi et al. (1973) attributed this to the longer length of liquid travel (730 vs. 150 ft.) in the cells.

The importance of organic loading on pathogen survival has been reported. Salmonella typhi was inoculated into simulated stabilization ponds at loading rates between 50 and 6000 lb BOD/acre/day. At 23C survival was 30 hours at 50 lb/acre/day, 2 days at 500 lb, and 11 days at 4000 lb (McGarry and Bouthillier, 1966). In another simulated pond with a 7-day detention period, a 100% die-away of Salmonella and Proteus was reported at loadings of less

than 85 lb/acre/day. At 212 lb/acre/day, this removal was reduced to 98.7%.

REMOVAL OF VIRUSES

As with pathogenic bacteria, the removal of viruses from wastewater by stabilization ponds has been studied by only a few workers. The majority of these investigations have been confined to observing the quantity of virus in the influent and effluent of ponds. Few have studied the mechanism of inactivation or removal or addressed the problem of how ponds might be designed to maximize virus reduction.

Four stabilization ponds in Israel were found to have an average of 290 enteroviruses/100 ml in the effluents (Kott, 1973). After an oral poliovirus immunization program in California, Englande et al. (1967) examined the efficiency of a maturation pond in the reduction of viruses. Over a 10-month sampling period 97% of 93 samples of the influent were positive for poliovirus, whereas, poliovirus was recovered from 19% of 87 effluent samples. Eight out of 141 samples were positive for enterovirus in the sewage entering a three-cell pond system in India, while no viruses were isolated from 96 effluent samples. Total detention time in these ponds was 4.8 days (Bopardikar, 1969). Slanetz et al. (1972) studied the effects of one-, two-, three-, and four-cell systems on the survival of indicator organisms and pathogenic agents in New Hampshire. They were able to isolate enteroviruses from many of the pond effluents throughout the year. In Israel, Shuval (1969) found that a four-cell system with a total detention time of 20 days had an average enteric virus removal efficiency of 67.5%. Malherbe and Strickland-Chomley (1967) studied a number of ponds in South Africa for their ability to lower virus concentrations in wastewater. In the effluent of the first pond, a four-cell system with a total holding time of 38 days, seeded poliovirus was recoverable 56 days after addition. The second system was a three-cell maturation pond with a total detention time of 7 days. This treatment reduced, but did not eliminate influent reo- and enterovirus levels. The third system consisted of a trickling filter followed by four maturation ponds in series with a 14-day detention time. Viruses were detected in eleven of 13 trickling filter effluent samples, but in only two of 16 pond effluent samples. Nupen (1970) studying this same facility, found a 95% reduction of total viruses and complete elimination of enteroviruses in the ponds (at least below their detention sensitivity). Christie (1967) was unable to detect seeded poliovirus Type III in a model stabilization pond after 14 weeks detention.

According to Slanetz et al. (1972) Malherbe and Coetzee (1965) state that the biological processes that treat wastewaters in stabilization ponds probably do not reduce virus levels. Therefore, they claim that adsorption to solids, the action of sunlight, or length of retention beyond normal virus survival times are the

likely mechanisms of virus removal in ponds. In contrast to this, however, Sobsey and Cooper (1971) found enhanced inactivation of poliovirus in both stabilization pond water and algae-bacterial mixed cultures over that seen in autoclaved pond water and bacterial cultures, respectively. They felt that the pH differences between tests and controls were too small to account for the differences in inactivation observed. Instead they attributed the differences to some unknown biological or heat labile factors in the test cultures. Bopardikar (1969) reported a study in which a Chlorella extract was responsible for poliovirus inactivation. Parker (1968), as cited in Young (1964), and Christie (1967) both suggest that adsorption to particulates and subsequent settling may account for some of the virus reduction observed in ponds.

ALGAE IN WASTE STABILIZATION PONDS

The process of waste treatment in stabilization ponds relies on the indigenous microbial population to reduce the complex organic wastes entering the ponds to simpler products. According to Oswald (1968), there are four major classes of reactions that occur in ponds. The first is aerobic conversion of carbohydrate into bacterial energy, carbon dioxide, and water. This is aerobic oxidation, and takes place in the presence of bacteria and oxygen:

$$6(CH_2O)_X + 5O_2 \rightarrow (CH_2O)_X + 5CO_2 + 5H_2O + \text{energy} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In the absence of free oxygen, conversion of carbohydrates to bacterial cell mass takes place with the formation of organic acids:

$$5(CH_2O)_X \rightarrow (CH_2O)_X + 2CH_3COOH + \text{energy} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The organic acids may then be converted to methane and carbon dioxide by methane bacteria:

$$5CH_3COOH \rightarrow 2(CH_2O)_X + 4CH_4 + 4CO_2 + \text{energy} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

If oxygen is present the organic acids may be oxidized as in equation (1). In the presence of sunlight, algae in the ponds convert carbon dioxide into complex organic matter and free oxygen:

$$(CH_2O)_X + CO_2 \xrightarrow{\text{(Light + Algae)}} 2(CH_2O)_X + O_2 + H_2O \quad . \quad . \quad . \quad (4)$$

Equations (1) and (4) are linked together because the bacteria depend on the algae for oxygen while algae depend on bacteria for the production of carbon dioxide and photosynthesis. Similarly equations (2) and (3) are linked as methane fermentation requires organic acids as a substrate (Oswald, 1968).

Although both aerobic and anaerobic decomposition may take place within a single pond, they are spatially separated. Anaerobic fermentation is confined to the lower stratum of the pond and within the sludge layer where there is no oxygen to interfere with the metabolism of methane bacteria. Aerobiosis exists near the surface where oxygen produced by algae is available for oxidative decomposition of waste by bacteria.

Both of these processes are important for the treatment of wastewaters. Anaerobiosis is most effective on concentrated wastes such as the settled solids at the bottom of a stabilization pond. The aerobic process is more efficient in reducing the dilute liquid waste above the sludge (Gloyna, 1971). Anaerobic decomposition, however, does give rise to odors and unsightly solids. Therefore, for the treatment of domestic sewage near population areas, it is important that aerobic decomposition be maintained within a pond for both practical and esthetic reasons.

It has been shown by many authors (e.g. Neel and Hopkins, 1956; Merz et al., 1957; and Neel et al., 1961) that atmospheric oxygen rarely, if ever, supplies enough oxygen to meet even a small portion of the aerobic decomposition-respiration demands within a pond. Therefore, without the continued presence and growth of phytoplankton, oxidative treatment of organic wastes would not continue. As algae are so important for the proper functioning of stabilization ponds, the factors that affect their concentration and distribution within a pond have received much study.

Seasonality is probably the most important environmental influence related to algal concentrations in stabilization ponds. Numerous studies have shown that the densities of algae are lower in ponds during winter than in summer (Caldwell, 1946; Neel and Hopkins, 1956; Merz et al., 1957; Parker, 1962; and Stouse, 1964). Most authors attribute this to the intensity of sunlight and not to temperature (Neel and Hopkins, 1956; Oswald and Gotaas, 1957; Bartsch, 1961; and Hodgson, 1964). Bartsch (1961) found in a study of seven Dakota ponds that an ice cover 26-66 centimeters thick could reduce surface light intensity from 1400 to 28 footcandles, which is less than the amount of light needed to meet algal respiration requirements. Neel and Hopkins (1950) found that phytoplankton densities were dependent on solar radiation, and during periods of ice cover they could disappear altogether. Towne et al., 1957, also determined that ice cover can effectively reduce light levels below those necessary for minimal algae respiration. That reduced algal concentrations in winter are a function of light intensity and not of low water temperatures has been demonstrated to be true by Towne et al., 1957; Neel et al., 1961; and Stouse (1964). In the early winter, before ice cover, algae and dissolved oxygen were still present in the stabilization ponds. After ice formation, the algae had disappeared and anaerobic conditions prevailed. However, Bartsch (1961) points out that low temperatures probably do slow down algal growth and certainly

reduce the biological degradation of wastes that provides algal nutrients. Therefore, with the approach of winter, algal metabolism and reproduction are reduced.

Organic loading rates also have an effect on algal concentrations. Mills (1961) and Stouse (1964) both found that algal populations became denser as loadings were increased up to 330 lb BOD/acre/day and 575 persons/acre, respectively. Oswald and Gotaas (1957) have determined that the dry weight of algal cells is a logarithmic function of organic loading up to 400 mg/l BOD. Neel et al., 1961, found that the production of algae increased up to 60 lb/acre/day BOD and then leveled off. They stated that at loadings above 40 lb/acre/day light intensity became the limiting factor in photosynthesis. Below this rate the limits were all due to nutrient availability. In contrast to these studies, Neel and Hopkins (1956) and Merz et al. (1957) could find no relationship between algal concentration and organic loading rate.

The extent to which sunlight can penetrate a pond will limit the depth at which algae can grow. Oswald (1968) reports that algae concentration is inversely proportional to pond depth. In Dakota stabilization ponds, 99% of the light was absorbed in the upper 50 to 70 centimeters (Bartsch and Allum, 1957). Within this layer, algal production of oxygen is greater than is the algal respiratory requirement. Bartsch (1961) states that there are three layers in every pond: an upper layer where incident light energy exceeds that required for photosynthesis; a layer below this at which photosynthesis is optimal; and finally, below this, a layer where both photosynthesis and the production of oxygen begin to decline. Since only a small portion of a pond harbors 99% of the incident solar energy (the euphotic zone), the result is a dramatic stratification of algae between the surface and bottom of the pond. Merz et al., 1957, report that during periods of good mixing, algal counts were 1.5 to 2 times as great on the surface as on the bottom of ponds in California. During one period, they found that the surface count was 15 times as great as the bottom count. Wachs and Berend (1968) have found most of the algae within 1.5 feet of the pond surface. They did state, however, that this stratification of algal cells was not as strong in winter. Finally, Hodgson (1964) reports, in an African study, that in comparing two ponds, one and three feet deep, the former always had higher algal concentrations. He attributed this to better lighting conditions in the first pond.

Detention time also has been found to have a pronounced effect on algal concentrations within ponds. Meron et al., 1965, in Israel noted that the number of algal cells began to decrease after 6.5 days of detention and continued to do so throughout the rest of the holding period. In Australia, Parker (1962) found that a combination of multiple cells and long detention times was useful in reducing algal concentrations in the final effluent.

The reasons for the progressive loss of algae with time are probably related to the concurrent reductions of BOD within ponds. Meron et al., 1965, felt that the algae became limited by the amount of carbon available as detention time increased. The quantities of ammonia nitrogen may also become limiting as algae assimilate this compound and as it is volatilized to the atmosphere (Young, 1974). It has also been found that the numbers of algal predators can increase with detention time (Hodgson, 1964), and subsequently may contribute to the removal of algae from stabilization ponds.

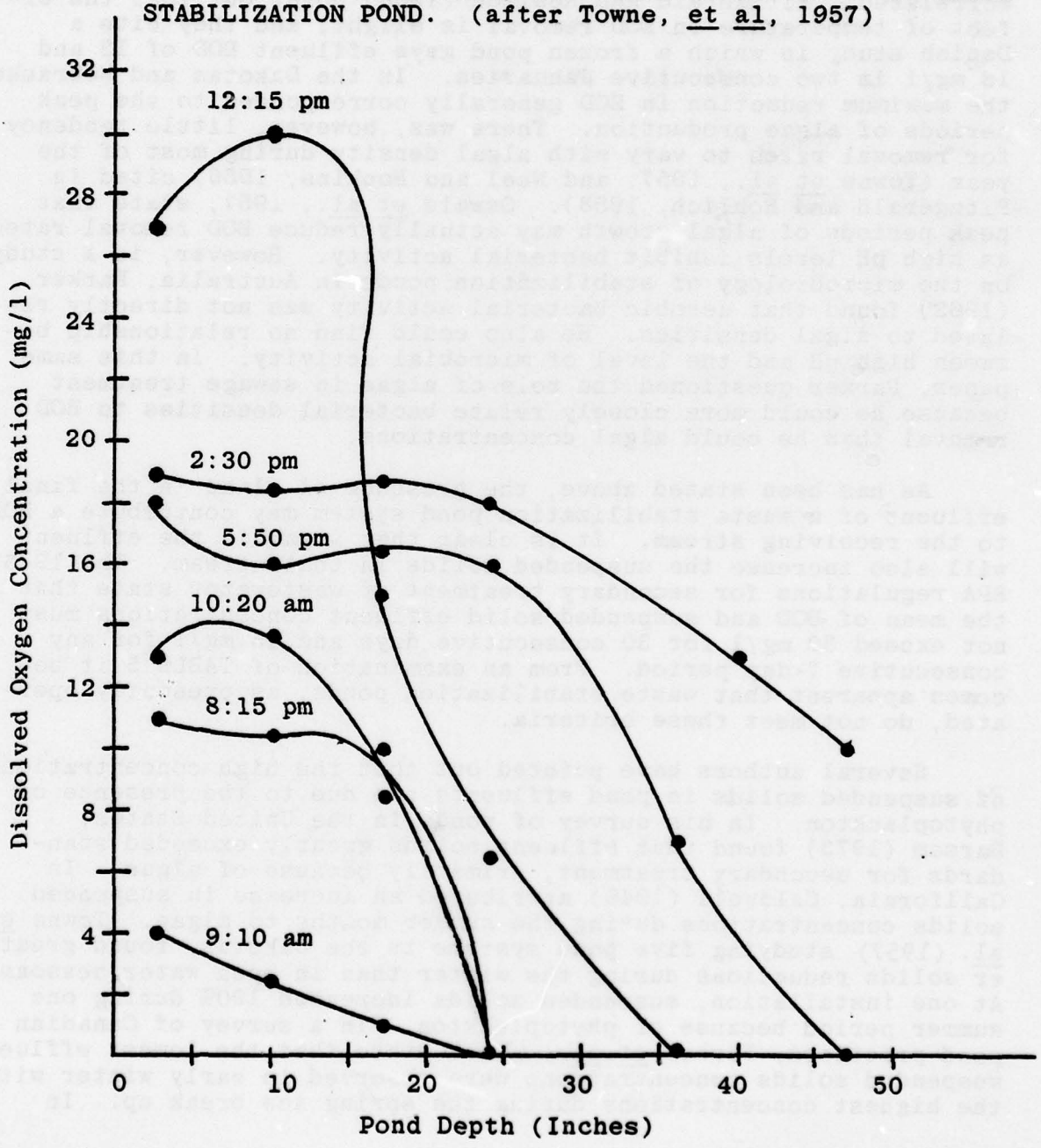
Neel et al. (1961) have pointed out that the activities of algae have almost "dictatorial" powers over pond biology and chemistry. Although it has been shown (Oswald and Gotaas, 1957) that algae do not participate directly in the reduction of organic wastes, their life processes do have pronounced influence on the movements and concentrations of many chemicals within the pond. Furthermore, as has been pointed out earlier, without their production of oxygen, aerobic decomposition of waste would not occur.

The release of oxygen by algae during periods of photosynthesis is their most important function as far as the removal of organic waste is concerned. It is obvious that oxygen production is dependent both on the distance from the pond surface and on the time of day. In a Dakota stabilization pond, oxygen production decreased rapidly with depth. The rate at the 24 and 38 inch depths was 14% and 3.5%, respectively of the oxygen production at 10 inches, whereas, respiratory use remained the same at all depths. At 32.5 inches, the 1% light level was reached, and oxygen production was half the rate of respiratory use. Oxygen release during the summer ranged from 17.8 lb/acre/hour at midmorning to 0.25 lb/acre/hour in the evening (Towne et al., 1957). The relationships of dissolved oxygen concentration with depth and time of day is presented graphically in FIGURE 6. Even though oxygen production near the surface of a pond may range from near saturation to several times saturation level (Towne et al., 1957), Oswald (1968) points out that the oxygen is used by bacteria at low efficiencies because the BOD is quickly removed from the surface by settling.

As photosynthesis is dependent on light intensity and temperature, dissolved oxygen concentrations also will be a function of season. Williford and Middlebrooks (1967) have shown that mean daily dissolved oxygen concentrations near the pond surface can be greater than 10 mg/l in the summer. During the winter, the concentration of dissolved oxygen is often less than 5 mg/l. Oswald et al.(1957) assert that the energy conversion from sunlight to oxygen seldom exceeds 10 to 12% of the available light energy. Therefore, a pond may produce several times as much oxygen in Summer as in Winter.

In Nebraska, Neel and Hopkins (1956) found that the decreased light intensities beginning in October slowed down photosynthesis to such an extent that the algae could not keep pace with the

FIGURE 6. DIURNAL CONCENTRATIONS OF DISSOLVED OXYGEN IN A DAKOTA STABILIZATION POND. (after Towne, et al, 1957)



decomposition-respiration demands of the ponds. The reduction in photosynthesis resulted in anaerobic conditions in the pond until the following April. It already has been pointed out that photosynthesis can be reduced or halted under ice. However, if loading rates are sufficiently light, aerobiosis can continue throughout the period of ice cover (Neel et al., 1961; Stouse, 1964).

It is apparent that the concentration of dissolved oxygen is directly related to photosynthetic activity, but it is not clear that BOD reduction and the rate of photosynthesis can be easily correlated. Fitzgerald and Rohlich (1958) point out that the effect of temperature on BOD removal is slight, and they cite a Danish study in which a frozen pond gave effluent BOD of 13 and 18 mg/l in two consecutive Januaries. In the Dakotas and Nebraska the maximum reduction in BOD generally corresponded to the peak periods of algae production. There was, however, little tendency for removal rates to vary with algal density during most of the year (Towne et al., 1957; and Neel and Hopkins, 1956, cited in Fitzgerald and Rohlich, 1958). Oswald et al., 1957, state that peak periods of algal growth may actually reduce BOD removal rates as high pH levels inhibit bacterial activity. However, in a study on the microbiology of stabilization ponds in Australia, Parker (1962) found that aerobic bacterial activity was not directly related to algal densities. He also could find no relationship between high pH and the level of microbial activity. In this same paper, Parker questioned the role of algae in sewage treatment because he could more closely relate bacterial densities to BOD removal than he could algal concentrations.

As has been stated above, the presence of algae in the final effluent of a waste stabilization pond system may contribute a BOD to the receiving stream. It is clear that algae in the effluent will also increase the suspended solids in that stream. The 1973 EPA regulations for secondary treatment of wastewater state that the mean of BOD and suspended solid effluent concentrations must not exceed 30 mg/l for 30 consecutive days and 45 mg/l for any consecutive 7-day period. From an examination of TABLE 5 it becomes apparent that waste stabilization ponds, as presently operated, do not meet these criteria.

Several authors have pointed out that the high concentrations of suspended solids in pond effluents are due to the presence of phytoplankton. In his survey of ponds in the United States, Barsom (1973) found that effluent solids greatly exceeded standards for secondary treatment, primarily because of algae. In California, Caldwell (1946) attributed an increase in suspended solids concentrations during the summer months to algae. Towne et al. (1957) studying five pond systems in the Dakotas, found greater solids reductions during the winter than in open water seasons. At one installation, suspended solids increased 190% during one summer period because of phytoplankton. In a survey of Canadian pond practices, Fisher et al. (1968) state that the lowest effluent suspended solids concentrations were observed in early winter with the highest concentrations during the spring ice break up. In

contrast to the above reports, Mackenthum and McNabb (1961) in Wisconsin, found that effluent suspended solids were generally lower in summer than in winter.

TABLE 5. EFFLUENT CONCENTRATIONS AND REDUCTION EFFICIENCIES OF SUSPENDED SOLIDS IN WASTE STABILIZATION PONDS

Location	Season	Effluent ss (mg/l)	Average % Reduction	Range of % Reduction	Reference
Mexico	F	101	88	--	De la Espino and Aguirre Martinez 1976
California	S	--	increase	--	Caldwell, 1946
Canada	all year	--	80	61-95	Fisher et al., 1968
Dakotas	S	--		1-66	Towne et al., 1957
Dakotas	W		--	68-93	Towne et al., 1957
Wisconsin	all year	225-570	--	--	Mackenthum and McNabb, 1961
California	W	46	83	--	Merz et al., 1957
California	Sp	62	70	--	Merz et al., 1957
Southwest USA	yearly	80	--	--	Barsom, 1973
South Central USA	yearly	110	--	--	Barsom, 1973
Southeast USA	yearly	100	--	--	Barsom, 1973
Ohio Basin	yearly	540	--	--	Barsom, 1973
Great Lakes Basin	yearly	40	--	--	Barsom, 1973
Middle Atlantic	yearly	110	--	--	Barsom, 1973
Australia	S	124	58	--	Parker et al., 1959
Australia	S	69	--	--	Parker et al., 1950
Australia	S	27	50	--	Parker et al., 1959
Australia	W	51	4.6	--	Parker et al., 1959
Canada	all year		70		Dawson & Grainge, 1969

HYDROGEN ION CONCENTRATION

The diurnal fluctuations in pH are a result of the carbon dioxide demands of algae during the periods of photosynthesis. The carbon dioxide produced by decomposition-respiration tends to lower the pH of the pond, while uptake of this compound by algae moves the pH towards alkalinity. Neel et al. (1961) state that pH levels below 8.0 indicate a failure of the phytoplankton to utilize all available CO_2. Levels above 8.0 indicate a CO_2 demand greater than the quantities furnished by decomposition-respiration. Data from Williford and Middlebrooks (1957) indicate that pH reaches its maximum during the late afternoon hours and remains high until after sunset. They have also shown that this cycle is confined to the surface of the pond where photosynthesis is most active.

Besides daily fluctuations in pH there is a seasonal cycle of hydrogen ion concentration. Williford and Middlebrooks (1957) have shown that daily pH values are lower during the winter months than at other times of more active photosynthesis. The pH difference between the top and bottom of ponds is not as pronounced during the cooler months as in summer because of reduced photosynthetic activity and greater mixing of the pond contents in the winter.

TABLE 6 indicates the effluent pH values that a number of investigators in different locations have found for various times of the year. It can be seen that maximum pH can vary as much as four pH units between summer and winter in a single pond, especially at high latitudes. The minimum pH encountered in the warm months does not drop much below 8.0, whereas, during the winter, the maximum can be near 7.0. The maximum pH values during the summer are well above 9.0 in every case listed. It should be pointed out, however, that many of these data probably have been gathered from near the pond surface. One would expect that near the bottom, anaerobic conditions will keep the pH low year-round.

The diurnal shift in pH is accompanied by a cyclical change in carbon dioxide (CO_2) concentration; CO_2 concentration drops off as algae assimilate carbon during photosynthesis. The amount of CO_2 in the pond rises at night as decomposition-respiration continues and photosynthesis is curtailed (Williford and Middlebrooks, 1967).

The free CO_2 extracted by algae raises the pond pH to about 8.3. The pH is increased above this level by extracting CO_2 from the bicarbonates with which CO_2 is removed, the concentration of carbonate increases, thus increasing the hydroxyl ion concentration and consequently raising the pH (Williford and Middlebrooks, 1967). Therefore, during the day there is a buildup of carbonate alkalinity and a decrease in the bicarbonate alkalinity. The former tends to precipitate out as $CaCO_3$ in the daylight hours only to be brought back into solution at night as $Ca(HCO_3)_2$ by decomposition-respiration (Neel et al., 1961).

TABLE 6. EFFLUENT pH VALUES IN STABILIZATION PONDS

Location	Season	Influent pH	Effluent pH Average	Effluent pH Range	Reference
Missouri	Sp	7.0-7.3	--	8.0-9.0	Neel et al., 1961
Missouri	S	6.8-7.2	--	8.6-10.5	Neel et al., 1961
Missouri	F	6.8-7.2	--	8.7-10.0	Neel et al., 1961
Missouri	W	7.0-7.2	--	7.8-8.8	Neel et al., 1961
Nebraska	all year	7.4-8.2	--	7.2-9.5	Neel and Hopkins, 1956
Texas	all year	--	9.0	8.1-9.8	Ullrich, 1967
Texas	all year	--	9.2	8.2-10.1	Ullrich, 1967
Texas	all year	--	9.2	7.8-9.8	Ullrich, 1967
Texas	all year	7.6	--	8.2-9.6	Warrington, 1952
California	all year	--	7.8	--	Merz et al., 1957
Wisconsin	W	--	--	7.0-7.5 Maximum	Mackenthum & McNabb, 1961
Wisconsin	S	--	--	10.0-11.0 Minimum	Mackenthum & McNabb, 1961
Wisconsin	W	--	--	6.9-7.1 Maximum	Mackenthum & McNabb, 1961
Wisconsin	S	--	--	9.0	Mackenthum & McNabb, 1961
Wisconsin	all year	--	--	7.7-9.3	Mackenthum & McNabb, 1961
Israel	NG	--	8.0	--	Meron, et al., 1965
Mississippi	S	--	--	8.5-10.0 Maximum	Williford & Middlebrooks, 1967
Mississippi	all year	--	--	9.7 Minimum	Williford & Middlebrooks, 1967
Mississippi	all year	--	--	8.5 Maximum	Williford & Middlebrooks, 1967
South Africa	W	--	--	9.0 Maximum	Drews, 1966
South Africa	S	--	--	>10.0	Drews, 1966
Florida	S	--	--	8.9-9.8	Mills, 1961
Africa	all year	--	--	7.2-8.8 Maximum	Hodgson, 1964
Africa	all year	--	--	9.6	Hodgson, 1964
Kansas	Sp	--	--	7.2-8.0	Loehr & Stephenson, 1965
Kansas	S	--	--	8.1-9.0	Loehr & Stephenson, 1965
Kansas	F	--	--	7.6-8.8	Loehr & Stephenson, 1965
Kansas	W	--	--	7.0-7.2	Loehr & Stephenson, 1965

NITROGEN

Although algae do not participate in the decomposition of organic wastes *per se*, these wastes serve as the substrate for algal growth and metabolism. It has already been seen that the utilization of CO_2 by algae affects the disposition of this compound in stabilization ponds. The other principal products of aerobic bacterial oxidation of organic wastes are nitrogenous compounds, primarily ammonia (Oswald and Gotaas, 1957). Nitrogen is the nutrient used in greatest quantities by algae and other plants. Therefore, the utilization of waste nitrogen by algae may be considered as a sink for this element in stabilization ponds. As stated by Fitzgerald and Rohlich (1958) very few nutrients are lost from ponds but rather are concentrated in algal cells. The ultimate fate of nitrogen and other plant nutrients depends on the fate of the algae.

TABLE 7 is a tabulation of nitrogen removal efficiencies from stabilization ponds over a large geographical area. The removals of ammonia-nitrogen are generally above 75%, but the wide range of values indicate differing abilities of ponds to remove nitrogen, probably due to the condition of the algal population within the ponds. In their literature survey, Fitzgerald and Rohlich (1958) found ammonia-nitrogen reductions of from 15 to 40 mg/l in the influent to 72 mg/l in the effluent. Nitrate- and nitrite-nitrogen concentrations were considered to be insignificant in relation to ammonia-nitrogen.

The metabolic rate of algae is dependent on factors such as temperature and light intensity. From this it would be expected that nitrogen reductions would be greater during the summer than in winter if algae are responsible for nitrogen removal in ponds. In a survey of Iowa stabilization ponds, Stouse (1964) found a definite increase in effluent nitrogen, as ammonia, during the winter. Median ammonia-nitrogen concentrations were 35 times as great in winter as in summer. He attributed this effect to a failure of algae to use this compound in sufficient quantities. In Wisconsin, Mackenthum and McNabb (1961) observed that the trends of BOD and total nitrogen reduction followed the same seasonal cycles. They felt that the decline in effluent quality in winter was due to a decrease in algae densities in these months. Seasonal variation in ammonia-nitrogen in pond effluents also has been noted by Caldwell (1946), Neet *et al*. (1961) and Bolitho and Dipl (1964).

Organic removal of nitrogen is evidenced by its changes in form through a stabilization pond. Caldwell (1946) and Stouse (1964) found that during the summer months, there was an increase in nitrate levels from influent to effluent. Stouse observed no effluent nitrate in the winter. They stated that high nitrate levels in stabilization pond effluent is indicative of oxidative decomposition of waste and of a high degree of treatment. Parker (1962) observed an increase in organic nitrogen through a series of stabilization ponds in Australia. He attributed this to algal

TABLE 7. PERCENT REDUCTION OF NITROGEN IN WASTE STABILIZATION PONDS

Location	Season	Organic N	NH_3 N	NO_2 N	NO_3 N	Total N	Reference
Oklahoma	all year	--	--	--	--	30-95	Assenzo & Reid, 1966
South Africa	all year	--	--	--	--	54	Bolitho & Dipl, 1964
South Africa	all year	--	65	--	20	77	Bolitho & Dipl, 1964
California	S	--	74	increase	increase	--	Fitzgerald & Rohlich, 1958
Germany	S	--	91	28	--	--	Fitzgerald & Rohlich, 1958
Germany	S	--	87	100	100	--	Fitzgerald & Rohlich, 1958
Texas	S	--	96	--	--	--	Fitzgerald & Rohlich, 1958
Denmark	S	--	63	--	--	--	Fitzgerald & Rohlich, 1958
California	S	--	93	increase	increase	--	Fitzgerald & Rohlich, 1958
Germany	S	--	97	no change	49	--	Fitzgerald & Rohlich, 1958
Australia	S	85	50	--	--	--	Fitzgerald & Rohlich, 1958
Germany	S	67	97	--	increase	--	Fitzgerald & Rohlich, 1958
USSR	S	--	89	--	no change	--	Fitzgerald & Rohlich, 1958
Canada	all year	--	75	--	--	70	Fisher et al., 1968
Missouri	all year	--	82	--	--	93	Neel et al., 1961
Missouri	Sp	--	96	--	--	--	Neel et al., 1961
Missouri	S	--	75	--	--	--	Neel et al., 1961
Missouri	F	--	98	--	--	--	Neel et al., 1961
Missouri	W	--	98	--	--	--	Neel et al., 1961
Michigan	all year	--	--	no change	--	--	Annett et al., 1974
Texas	all year	--	95	--	--	--	Ullrich, 1967
Nebraska	all year	--	23	50	no change	--	Neel & Hopkins, 1956
Australia	all year	42	43	--	--	--	Neel & Hopkins, 1956

development in the ponds. Similarly, in Wisconsin, ammonia comprised 80% of the total nitrogen in ponds under ice. At other times, organic nitrogen formed the bulk of the total nitrogen (Mackenthum and McNabb, 1961). Although these transformations of nitrogen were attributed to phytoplankton, Neel et al. (1961) found that organic nitrogen removal in Missouri ponds was 8 to 10% greater than ammonia-nitrogen. Since ammonia is the principal source of nitrogen for algae in stabilization ponds (Oswald and Gotaas, 1957), Neel et al. suggest that the level of organic nitrogen is lowered by bacteria, and ammonia is utilized by algae. This hypothesis was substantiated by Assenzo and Reid (1966) in Oklahoma, who concluded that algae were no more important in nitrogen removal than bacteria.

Although algae may be the major way that nitrogen is concentrated from stabilization pond waters, there are other means by which this element may be reduced in the effluent. Ammonia entering a pond is in equilibrium between its gaseous and hydroxyl forms:

$$NH_3 + H_2O \rightleftharpoons NH_4 + OH^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The reaction is very pH dependent, acidic or neutral pH driving the reaction to the right, and alkaline pH favoring gaseous ammonia (Straton, 1969). It has been shown above that during much of the year, pond waters can be quite alkaline, so at these times atmospheric loss of gaseous ammonia may be an important means of reducing nitrogen levels in ponds. Unfortunately, no empirical studies have been done on the rates of loss of gaseous ammonia from waste stabilization ponds. However, according to Young (1974), Folkman and Wachs (1972) have investigated ammonia loss from the surface of lime-treated sewage. They found that in addition to high pH, the volatilization of ammonia was dependent on wind velocity, pond depth, temperature, and mixing conditions within the ponds.

Johnson (1968) has stated that sediments and sedimentation are also responsible for nitrogen reductions in stabilization ponds. Seepage of nitrates into anaerobic pond sediments results in denitrifaction and subsequent release of gaseous nitrogen. Ammonia can be absorbed onto the soils beneath the sludge and remain there as long as anaerobiosis persists (Johnson, 1968). According to Young (1974), Foree and McCarty (1968) state that nitrogen in sedimented algae can be reintroduced into the overlying waters by anaerobic decomposition of the plant material and release of ammonia. They determined, however, that much of the algal-associated nitrogen remained in the pond sediments.

PHOSPHORUS

Like nitrogen, phosphorus is an essential nutrient for most forms of life including algae and bacteria. Unlike nitrogen,

however, phosphorus is not oxidized or reduced to a gas that can be eliminated from stabilization ponds to the atmosphere. Therefore, the quantities of phosphorus will be conserved in a pond and will undergo cyclical conversions from the organic to inorganic form as this element is used by algae and bacteria. Removal from the system is only possible through physical separation or complete insolubility (Barth, 1968; cited in Young, 1974).

The major mechanisms of phosphorus removal or concentration from waste stabilization pond waters include metabolic uptake of phosphorus by algae and bacteria and precipitation of phosphates at high pH.

An examination of TABLE 8 indicates that phosphorus removal efficiencies from stabilization ponds are very erratic. From their literature survey, Fitzgerald and Rohlich (1958) found that ponds removed an average of 40% of the influent phosphorus. Loehr and Stephenson (1965) concluded that stabilization ponds could not be relied on either for nitrogen or phosphorus removal from secondarily treated wastewaters. Despite the fact that phosphorus reduction in ponds is not consistent, the few available reports do indicate some removal of this nutrient in most cases.

The biological uptake of phosphorus can exceed that which is necessary for normal metabolism. In continuous algal culture experiments, Borchardt and Azad (1968) found that at phosphate levels of 0 to 1.5 mg/l (0 to 3%), the growth of algae was strictly dependent on the phosphate concentration. From 1.5 to 4.5 mg/l (3 to 9%) phosphate, the algae were able to store phosphorus above the critical level needed for maximum growth. Above a 9% concentration, additional phosphate was left in the substrate. The point at which phosphorus storage begins is when the phosphate concentration of the cell mass exceeds 3% on a dry-weight basis. They suggest that by manipulation of algal densities until they fall within this storage zone, phosphate removal could be maximized if some means of algal harvesting is employed.

Bacteria are also capable of storing phosphorus above the levels needed for metabolism (Borchardt and Azad, 1968). In fact, nutrient removal by bacteria may be more rapid than by algae, since the former have a higher reproductive rate than the latter. According to Young (1974), Morgan (1972) states that normal bacterial growth takes place when cellular phosphorus levels are 1% by dry weight. If influent phosphorus levels are between 1 and 1.6% of the bacterial cell mass, bacteria are able to store all incoming phosphorus. From a comparison of seven Oklahoma ponds, Assenzo and Reid (1966) concluded that algae were no more important than bacteria in phosphate removal from ponds. Since bacteria are able to store phosphorus, and since they have a more rapid life cycle than algae, it appears that bacteria may play an important role removal of this nutrient from stabilization ponds.

The rate of algal or bacterial metabolism largely determines the rate of biological uptake of phosphorus. Bogan (1961) has

TABLE 8. PHOSPHORUS REMOVAL EFFICIENCIES IN WASTE STABILIZATION PONDS

Location	Season	% Reduction			Reference
		Phosphate	Organic P	Total P	
Africa	all year	reduced	--	--	Hodgson, 1964
Oklahoma	all year	--	--	30-95	Assenzo and Reid, 1966
New York	all year	reduced	--	--	Nemerow and Bryson, 1963
Nebraska	all year	8	--	--	Neel and Hopkins, 1956
Wisconsin	all year	--	--	reduced	Mackenthum and McNabb, 1961
Michigan	all year	--	--	71	Annett et al., 1974
Texas	all year	83	--	--	Ullrich, 1964
Kansas	all year	--	--	0-33	Loehr and Stephenson, 1965
Canada	all year	--	--	60	Fisher, et al., 1968
South Africa	all year	-44 to +37	--	--	Gaillard and Crawford, 1964
Australia	not given	52-92	--	--	Simmonds, 1973

shown that the metabolic uptake of phosphorus increases as algal density and growth rate increase. Laboratory studies by Borchardt and Azad (1968) also point out the dependence of phosphorus removal on algal concentrations. The growth rate and density of algae are, in turn, affected by temperature, which accounts for the fact that the greatest reductions in phosphorus levels in ponds are generally seen during the summer months (Fisher et al., 1968; Mackenthum and McNabb, 1961; Loehr and Stephenson, 1965; Simmonds, 1973).

Although algae and bacteria may play a role in phosphorus removal from ponds, they are not the sole means by which the levels of this nutrient can be reduced. Under the conditions of high pH, phosphorus readily forms insoluble complexes that can settle to the bottom of a pond. The cation that is most available in domestic sewage for the formation of this insoluble complex is Ca^{++}. Bogan (1961) found that calcium ion concentration and pH are the principal controlling factors in phosphate solubility.

Insolubility of phosphate depends on a pH above 9.0, and as the pH becomes lower due to diurnal or seasonal cycles or pond stratification, dissolution of insoluble phosphorus may occur.

Gaillard and Crawford (1964) found that as pH dropped, effluent phosphate levels became greater than influent concentrations. According to Young (1974), Hemens and Stander (1968) found considerable dissolution of phosphate during the night. It should be noted that although the chemical coagulation of phosphate is not effected directly by algae, the growth rate of phytoplankton indirectly controls phosphate precipitation by controlling the pH of the stabilization pond.

SLUDGE LAYER

Although the majority of research on waste stabilization ponds has been concerned with conditions that maximize waste reduction in aerobic portions of the pond, the role of the sludge layer in waste treatment must not be undervalued. Digestion of organic material in pond sludge is an important factor in reducing BOD since after 6 to 8 hours as much as 50% of the influent BOD may be deposited here (Meron et al., 1965). The interaction of sludge layer with the overlying water also needs to be understood better as it has been shown that coliforms persist longer under anaerobic than aerobic conditions (Marais, 1966) and polioviruses can be recovered from sludge long after they have disappeared from the pond water (see below).

Digestion of organic matter in the sludge takes place in the absence of oxygen. The first phase of anaerobiosis is the breakdown of carbohydrates, proteins and fats into simple alcohols and acids, mainly acetic, propionic and butyric acids (Brockett, 1976). A simplified version of this reaction has already been presented in equation (2). The organisms responsible for the conversion of complex organic molecules to volatile organic acids are anaerobic cellulolytic bacteria such as B. cellulosae dissolvens and the "lypolytic" Pseudomonas group (Parker et al., 1963). The acids and alcohols produced by this first group of organisms then serve as substrate for methane fermentation by organisms such as Methanobacterium omelianski and Mb. foricum (Parker et al., 1963). The simplified form of this reaction has been presented in equation (3). The effective range of temperature for volatile acid formation in pond sludges is between 4 and 40C, maximal acid production occurs at 25C. The optimal pH for these reactions is 6.5, with a range between 4.3 and 7.5 (Oswald, 1968). Methane fermenting bacteria are more sensitive to changes in temperature and pH than are volatile acid formers. Sludge temperatures must be between 15C and 40C (optimally 32C) for methane production, with a pH between 6.8 and 7.2 (optimally 7.0) (Oswald, 1968).

After the volatile acids are formed, they may move into the overlying water, or may be converted to methane, or may be oxidized to inorganic compounds by sulfate-reducing bacteria if sulfate is available (Foree and McCarty, 1970). Foree and McCarty state that the production of sulfides proceeds before methane fermentation if sulfates are available in sufficient quantity. This reaction is undesirable, however, as sulfides can cause odor problems in

stabilization ponds, and being more soluble than methane, can exert an additional BOD if they move into the aerobic zone.

Volatile acids also can move into the aerobic zone before being converted to methane or sulfides, resulting in an additional oxygen demand on that portion of the pond. McKinney et al. (1971) estimate that 10 to 20% of the settled BOD eventually will undergo aerobic decomposition. Gloyna (1968) points out that the rapid digestion in the sludge layer during the summer often results in vigorous bubbling and the formation of sludge mats on ponds, thus increasing the BOD at the surface. Parker et al. (1950) feel that the movement of anaerobic bacteria into the oxygen depleted waters adjacent to the sludge may contribute significantly to organic removal in stabilization ponds.

According to Young (1974), Foree and McCarty (1968) state that solids deposition in the sludge layer is due to suspended solids from the influent sewage and bacterial and algal particulates that have been synthesized in the pond. The mechanisms of sludge deposition include: physical sedimentation of particulates, bioflocculation of algae and bacteria, autoflocculation of solids by precipitation with metallic salts at high temperatures and pH, and sedimentation of feces by pond invertebrates.

Middlebrooks et al. (1965) and Oswald (1968) state that solids settle out rapidly near a stabilization pond inlet. Oswald cites a Woodland, California pond in which the deposition of sludge near the central inlet exceeded 9000 lb/acre/day at an areal loading of only 50 lb BOD/acre/day. He found in pilot plant studies that solids deposition at the inlet could be reduced by recirculation.

Accumulation of solids at the pond entrance can result in vigorous anaerobic digestion in this zone. Rising gas bubbles will buoy up mats of sludge that may be deposited in other areas of the pond. Hodgson (1964) found accumulations of sludge on the leeward side of African ponds. Middlebrooks et al. (1965) could find no significant effects of wind direction on the accumulation of sludge. However, they and Oswald et al. (1959) (cited in Middlebrooks et al., 1965) did observe considerable solids deposition in the corners of ponds. Middlebrooks et al., felt these deposits were due to sludge accumulation at the pond entrance, buoying of solids to the surface by rising gases and movement of these solids to the corners. Marais (1966) also observed the formation mats at the surface of stabilization ponds, especially during the summer. He stated that the critical sludge temperature for this phenomenon was 22C.

Another factor that may affect the sludge profile in a pond is the relative temperature of the incoming sewage and pond waters. If the influent is warmer than the water, the sewage tends to spread out over the pond surface and disperse the solids. However, if the sewage is cooler than the pond, then the solids will move

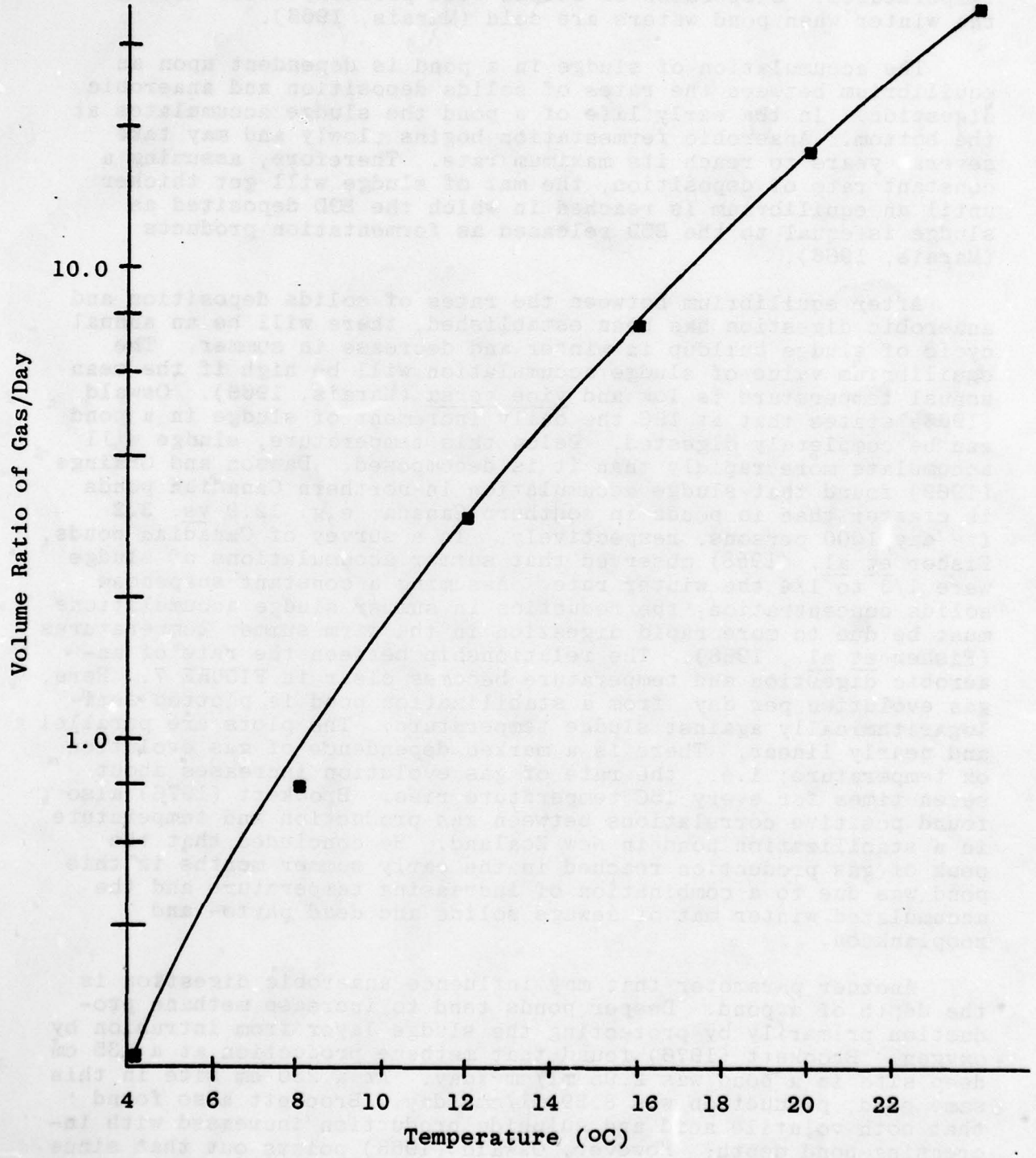
quickly to the bottom with greater accumulation near the inlet (Oswald, 1968). The latter phenomenon is more likely to occur in summer when intense solar radiation results in high pond surface temperatures. Dispersion of solids will probably occur during the winter when pond waters are cold (Marais, 1966).

The accumulation of sludge in a pond is dependent upon an equilibrium between the rates of solids deposition and anaerobic digestion. In the early life of a pond the sludge accumulates at the bottom. Anaerobic fermentation begins slowly and may take several years to reach its maximum rate. Therefore, assuming a constant rate of deposition, the mat of sludge will get thicker until an equilibrium is reached in which the BOD deposited as sludge is equal to the BOD released as fermentation products (Marais, 1966).

After equilibrium between the rates of solids deposition and anaerobic digestion has been established, there will be an annual cycle of sludge buildup in winter and decrease in summer. The equilibrium value of sludge accumulation will be high if the mean annual temperature is low and vice versa (Marais, 1966). Oswald (1968) states that at 19C the daily increment of sludge in a pond can be completely digested. Below this temperature, sludge will accumulate more rapidly than it is decomposed. Dawson and Grainge (1969) found that sludge accumulation in northern Canadian ponds is greater than in ponds in southern Canada, e.g. 12.9 vs. 3.2 ft^3/day/1000 persons, respectively. In a survey of Canadian ponds, Fisher et al. (1968) observed that summer accumulations of sludge were 1/3 to 1/4 the winter rate. Assuming a constant suspended solids concentration, the reduction in summer sludge accumulations must be due to more rapid digestion in the warm summer temperatures (Fisher et al., 1968). The relationship between the rate of anaerobic digestion and temperature becomes clear in FIGURE 7. Here, gas evolution per day, from a stabilization pond is plotted semi-logarithmically against sludge temperature. The plots are parallel and nearly linear. There is a marked dependence of gas evolution on temperature; i.e., the rate of gas evolution increases about seven times for every 15C temperature rise. Brockett (1976) also found positive correlations between gas production and temperature in a stabilization pond in New Zealand. He concluded that the peak of gas production reached in the early summer months in this pond was due to a combination of increasing temperature and the accumulated winter mat of sewage solids and dead phyto- and zooplankton.

Another parameter that may influence anaerobic digestion is the depth of a pond. Deeper ponds tend to increase methane production primarily by protecting the sludge layer from intrusion by oxygen. Brockett (1976) found that methane production at a 135 cm deep site in a pond was 2.95 ml/cm^2/day. At a 230 cm site in this same pond, production was 8.29 ml/cm^2/day. Brockett also found that both volatile acid and sulphide production increased with increasing pond depth. However, Oswald (1968) points out that since the temperature lapse rate in 10 to 12 feet deep ponds may average

FIGURE 7. GAS EVOLUTION FROM THE SLUDGE LAYER AS A FUNCTION OF TEMPERATURE. (after Marais, 1966)



1C/ft in the summer, cool temperatures at the bottom may inhibit sludge digestion.

The accumulation of sludge at the bottom of stabilization ponds has been studied by several workers. After five years of operation with an average BOD loading of 187 lb/acre/day, an Indian pond had only an average of 1.5 inches of sludge (Kharkar and Tiwari, 1963). Middlebrooks et al. (1965) determined that the rate of sludge accumulation in Mississippi was only one foot per 27.6 years. Parker et al. (1963) stated that with areal loadings as great as 1000 lb BOD/acre/day, desludging a pond may be necessary only once every 10 years. Hopkins and Hopkins (1963) cite a Missouri study in which it was concluded that sedimentation due to organic matter would not impair pond performance for more than 100 years. Silting, however, can result in the loss of one foot of pond depth every 25 years. This conclusion was restated by Middlebrooks et al. (1965) who determined that about 73.5% of the influent sludge to a pond can be composed of silt and other inorganic matter.

TEMPERATURE

It is felt generally that temperature is the most important single factor in the treatment of waste in stabilization ponds. Removal of BOD and indicator organisms both are influenced by this parameter. The temperature of a stabilization pond is largely uncontrolled by design and usually closely follows ambient conditions. However, as incident solar energy is confined to the upper portion of ponds, these, like other bodies of water, undergo a phenomenon termed thermal stratification. Stratification is most common during the mild and hot seasons, and is the development of a layer of warm water above and cool water below a thermocline. It is caused by the differential heating and differential densities of water (Oswald, 1968).

There are several factors that influence the development of thermal stratification in stabilization ponds. The most important of these is the depth of light penetration, which in turn is most affected by turbidity. High turbidity and low light penetration are most favorable for stratification (Stahl and May, 1967). Bartsch and Allum (1957) state that the penetration of light in waste stabilization ponds is much less than in most bodies of water due to the dense populations of algae of such ponds. In a Dakota study they found that at no time did the euphotic zone (the depth at which 1% of the incident light remains) extend beyond 0.7 meters. During the summer at a relatively light loading of 23 lb BOD/acre/day, Bartsch and Allum found the euphotic zone was 0.1 meters deep. In contrast, at a nearby reservoir, the depth of light penetration was seven meters. Because solar energy is confined to the very upper portion of ponds, especially during periods of dense algae populations, the differences in temperature between the top and bottom of stabilization ponds can be as great as that found in much deeper lakes (Stahl and May, 1967).

Stahl and May (1967) point out that as ponds contain small volumes of water, diel changes in light and temperature can influence stratification. Marais (1966) lists the mixing changes in a pond that results in diel temperature cycles:

1. In the early morning, there is a period of complete mixing during which temperature becomes the same throughout the pond.

2. At some point (usually in the early afternoon) a sharp thermocline develops with the temperature of the upper layer reaching a maximum and then gradually decreasing. The temperature of the lower layer falls rapidly until it is approximately the same as the earth and then remains constant.

3a. Under quiescent wind conditions, the layers above the thermocline cool more rapidly than those below as solar radiation decreases. Therefore, surface temperature decreases uniformly. The thermocline sinks and mixing ensues when the water temperatures above and below it are equal.

3b. Under windy conditions, in a period of decreasing temperatures, there is a gradual mixing of the waters adjacent to the thermocline, displacing it downward and eventually mixing the pond.

A study by Stahl and May (1967) in the state of Washington illustrates this daily temperature pattern. There was a steady decrease in temperature at the one cm level from 1330 hours to 0530 hours. As the upper portion of the water cooled in the late afternoon, a period of mixing was initiated from 1930 to 0130 hours. From this time until 0930, the pond temperature was quite uniform throughout except at 0530 when the surface cooled. At 0930, with the absorption of solar energy at the pond surface, stratification again began to develop in the pond. The diel temperature range at the one cm depth was 18.3C, at ten cm, 10.3C, at 30 cm, 2.2C, and at 50 cm, 0.4C. Stahl and May found little increase in temperature after 1330 hours and the maximum rate of cooling occurred just after sunset. Over a 2 year period of observation in Zambia, Marais (1966) found that diel variations at the bottom of a stabilization pond rarely exceeded 1C, whereas, variations at the surface ranged from the maximum recorded to that of the bottom.

Since ambient temperature varies seasonally as well as daily, it is not surprising that the pattern of pond heating and cooling will differ in the warm and cold months. Wachs and Berend (1968) observed thermal gradients during the summer, but not during winter in stabilization ponds in Israel. During the winter, there was enough solar radiation to heat the pond surface, but this only penetrated to 0.5 meters, below which the pond temperature was fairly uniform. In the summer, there was a marked thermal gradient of about 11C from surface to bottom. At night, the surface cooled

down but the gradient still existed. No such early morning gradient could be discerned in this pond during the winter.

Other climatic factors that can influence pond temperature are cloud cover and wind. Stahl and May (1967) found that cloudy conditions could depress pond temperatures by as much as 10 to 15C during the summer. They also found that the least amount of thermal stratification occurred on days that were cool, cloudy, and windy. This is due, in part, to less incident solar energy at the pond surface, and, in part, to greater mixing of the pond waters. In Dakota ponds, Towne et al. (1957) observed that thermal gradients did not develop if the ponds were subjected to mixing caused by windy conditions. Stahl and May (1967) found that the temperature lapse from the surface to bottom of a 50 cm deep pond was maximal on clear, calm days. The lapse rate was minimal on overcast,windy days. Marais (1966) states that if wind speeds are low in the summer, cooling by radiation from the surface may not be sufficient for mixing of the pond contents and the thermocline may persist.

Design criteria that affect the thermal conditions within a pond are depth and surface area. Stahl and May (1967) state that deeper ponds and ponds with small surface areas are more likely to be stratified than shallow ponds or those with large surface areas. Oswald (1968) has shown that shallow ponds accumulate heat more rapidly than deep ponds, but they also radiate heat away at a faster rate during the night. Therefore, deeper ponds have lower average temperatures, but the diel temperature is more uniform. Stahl and May (1967) point out that back radiation from the surface of a pond is more rapid if that surface contains a great deal of heat. This means that stratified ponds will lose heat more rapidly than unstratified ponds.

The different densities of the upper and lower layers of water brought about by differential heating also result in stratification of dissolved oxygen, pH, alkalinity and conductivity (Stahl and May, 1967). Although oxygen stratification exists in ponds during both summer and winter, Marais (1966) points out that the high rate of digestion in summer is likely to make the entire pond turn anaerobic after sunset. This is not as likely to occur in winter when low temperatures reduce the rate of digestion.

Marais (1966) states that the majority of the day's influent will enter a pond during the period of stratification. During the hot and mild months the temperature of the influent sewage normally will be cooler than that of the pond waters. Therefore, during these months most of the influent will have a tendency to sink rapidly to the lower regions of the pond. Marais found that this phenomenon had an effect on E. coli distribution in an African pond in the summer. Surface coliform counts were consistantly higher during the period of mixing than during the period of stratification. He also found higher effluent coliform counts in the pond effluent during summer than in winter. Marais speculates

that the anaerobic conditions near the pond bottom may account for the differences in coliform distribution and survival.

MATERIALS AND METHODS

MODEL PONDS

The model ponds used in this study were constructed of cast concrete tanks five feet in diameter. The tanks for the 18-, 30-, and 42-inch ponds were four feet deep and of single-piece construction; those for the 90-inch ponds were made by stacking two of these tanks and sealing the junction. In order to obtain 18- and 30-inch depths in the shallow ponds, these tanks were backfilled with a combination of pea gravel and sand to depths of 24 and 12 inches, respectively. Three ponds of each depth were built for a total of 12 ponds.

A 40-foot by 30-foot area was excavated at Balcones Research Center, Austin, Texas. The 12 tanks were placed in the excavation site approximately four feet apart. Soil was then backfilled around the tanks to within 12 inches of the top of each. The field site and situation of the model ponds are illustrated in FIGURE 8. The construction and placement of these ponds took place between June and August, 1975.

In order to minimize virus interaction with the tank and its leachates and to minimize leakage, the ponds were lined with six mil plastic sheeting. Before lining the ponds, a laboratory test was performed to determine the degree to which poliovirus might attach to the plastic. Two 2-liter beakers were filled with deionized water and two others were filled with dechlorinated final effluent. A piece of plastic liner was placed in one of the beakers with deionized water and one with final effluent; the other two served as controls. Poliovirus 1 (Chat) was added to each of the four beakers to approximately 6×10^4 pfu/ml. The volumes were continuously mixed with magnetic stirrers at room temperature (20-23C) and sampled over an 18-hour period. As it can be seen in FIGURE 9, recovery of infectious units is nearly identical in both the tests and controls, indicating little attachment to the plastic or differential inactivation. As it developed, the liners leaked and were cumbersome and awkward to handle. Therefore, at the end of the initial field test the plastic was removed from the ponds and they were sealed with Thoroseal® (dry mix). The Thoroseal® is prepared with ACRYL-60 and is used as a swimming pool sealant, routinely. The effects of the Thoroseal® on poliovirus were evaluated in the same way as the plastic liner, with a small piece of Thoroseal® coated concrete replacing the liner. Results of this study, shown in FIGURE 10, indicate that neither viral attachment nor viral inactivation occurs as a result of using the sealer.

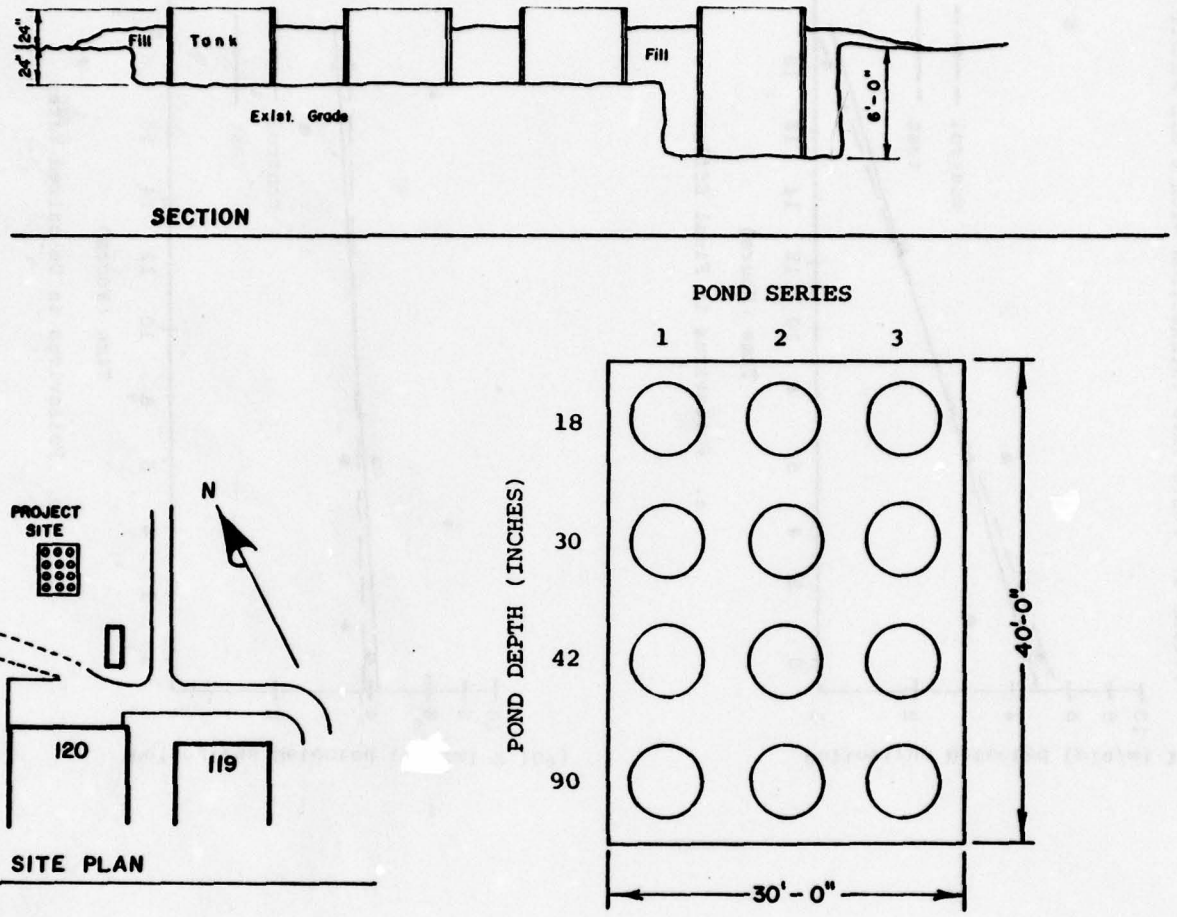
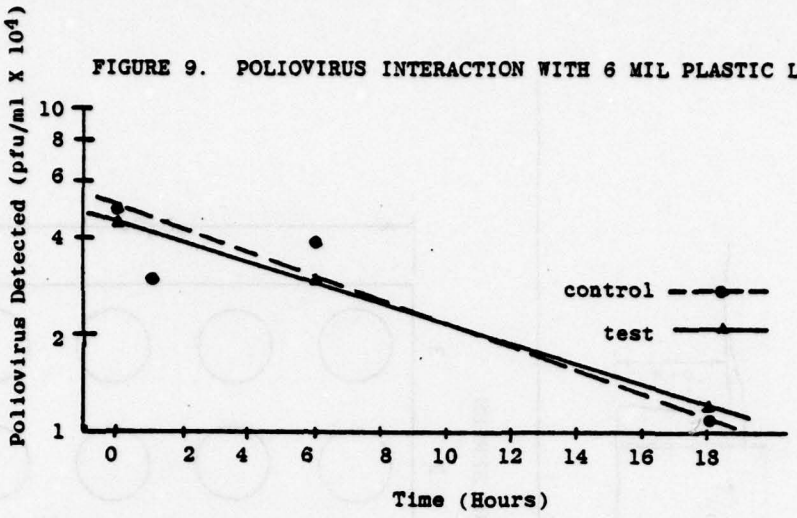
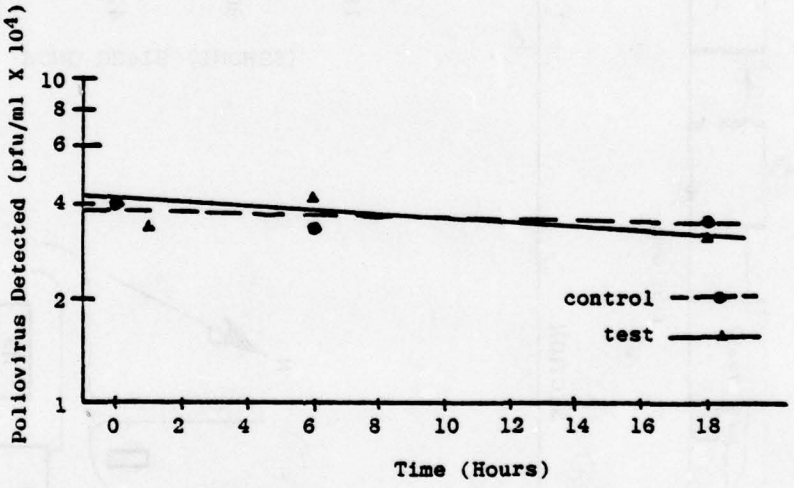


FIGURE 8. FIELD SITE AND SITUATION OF MODEL WASTEWATER HOLDING PONDS, BALCONES RESEARCH CENTER, UNIVERSITY OF TEXAS AT AUSTIN.

FIGURE 9. POLIOVIRUS INTERACTION WITH 6 MIL PLASTIC LINER.

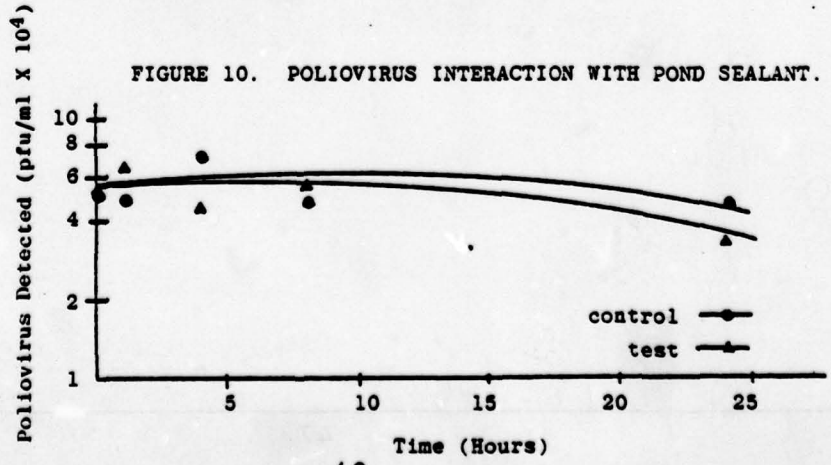


a. Poliovirus in Final Effluent



b. Poliovirus in Deionized Water

FIGURE 10. POLIOVIRUS INTERACTION WITH POND SEALANT.



Throughout the study period considerable difficulty was experienced in eliminating leaks from the ponds. As a consequence of this problem only two ponds of each depth were used throughout this study.

WASTEWATER

All wastewater for this study was obtained from Govalle Wastewater Treatment Plant, a component of the Austin, Texas, municipal wastewater treatment system. This plant employs the contact-stabilization process with a 20-to 30-minute contact period and a 4-hour stabilization period.

The types of wastewater used in this study were defined as primary effluent and final effluent. Primary effluent was raw sewage which had undergone settling for 30 minutes. Final effluent was obtained after wastewater was exposed to secondary treatment and disinfection with chlorine.

Both wastewaters were trucked to the model pond field site using commercial septic tank service vehicles. The day before the commencement of a field test, the holding tank of the transport truck was thoroughly rinsed. First the final effluent was transported to the field site where the appropriate number of holding ponds were filled, then the primary effluent was hauled to the site and discharged into the remaining ponds.

GROWTH OF TEST VIRUSES

The poliovirus 1 (Chat) stocks used to seed the model ponds were grown in HeLa cells (calf serum adapted, Flow Lab). Subconfluent HeLa monolayers were infected at a multiplicity of infection (MOI) of 10. Maximum viral harvests were obtained by three cycles of rapid freeze-thaw at 8-10 hours post-infection. Cell debris was removed by centrifugation at 12,000 x g for 15 minutes. The resulting viral stocks, average titer 1 to 3 x 10^9 pfu/ml, were stored at 4C until use.

The Coxsackievirus B3 (Nancy) used in this study was obtained from Dr. Charles Gauntt of The University of Texas Health Science Center at San Antonio. The virus stock was grown and stored in the same manner as was the poliovirus, except that the Coxsackievirus was harvested 20-24 hours after infection.

VIRUS ASSAY

Poliovirus assays were performed by inoculating the HeLa cell monolayers, on 3-60 mm plates, with 0.2 or 0.3 ml of sample. Virus inocula were adsorbed for 45 minutes at 37C with periodic rocking of the plates. The infected cells were overlaid with 4 ml of Eagles medium (modified) containing one percent Bacto-agar, 10

percent bovine serum, and antibiotics including penicillin, streptomycin, Gentamicin®, and Fungizone®. After incubation at 37C in 5.0% CO_2 for 2 days, monolayers were stained using 2% neutral red in Hanks' Balanced Salt Solution, and plaque-forming units (pfu) enumerated.

The procedure for infection of HeLa cell monolayers in the Coxsackievirus assay was identical to that used for the poliovirus. The overlay used for Coxsackievirus was similar to that used for poliovirus except that 2% bovine serum and 0.1 mg/ml DEAE-dextran were added to the medium. Plaques were observed by staining with neutral red 48 hours after infection.

In order to monitor both viruses simultaneously in the model holding ponds, it was necessary to neutralize the poliovirus before assaying the samples for Coxsackievirus. Lyophilized poliovirus 1 antiserum (rabbit) at a titer of 1:1200 was purchased from Microbiological Associates, Inc., of Bethesda, Maryland. The efficacy of this antiserum in neutralizing poliovirus in the presence of Coxsackievirus was tested in a single experiment. The rehydrated antiserum was diluted 1:50 by adding 0.02 ml of antiserum to 1.0 ml test volumes of virus. These volumes were mixed and incubated at 35C for 30 minutes. Infectivity (plaque) assays were done using HeLa cell monolayers. The results presented in TABLE 9 show virtually complete (>4 $\log_{10}$) neutralization of poliovirus, with no loss of infectivity for the Coxsackievirus. There was a loss of Coxsackievirus B3 in the presence of polio plus antiserum. However, results are reported as a percentage of the virus present at time 0. As the antiserum was used throughout the field test, it was not necessary to correct the data for inactivation due to the presence of the antiserum.

TABLE 9. THE EFFECT OF LYOPHILIZED POLIOVIRUS 1 ANTISERUM ON THE INFECTIVITY OF COXSACKIEVIRUS B-3 AND POLIOVIRUS I (CHAT)

Virus	- antisera	+ antisera
Coxsackievirus B-3	3.2×10^9	3.2×10^9
Poliovirus I (Chat)	2.1×10^9	$< 2.1 \times 10^5$
Coxsackievirus B-3 + Poliovirus I (Chat)*	2.7×10^9	1.8×10^9

* 0.5 ml of Coxsackie B-3 @ 3.2×10^9 pfu/ml and 0.5 ml of poliovirus I (Chat) @ 2.1×10^9 pfu/ml.

FECAL COLIFORM ASSAY

The presence of fecal coliforms in samples was determined by using either the standard membrane filtration technique for water or MPN determination for sediment. Both of these procedures were done in accordance with Standard Methods (APHA, 1976).

CHEMICAL/PHYSICAL ANALYSIS

Routine chemical analysis on field samples included: total suspended solids (TSS), volatile suspended solids (VSS), total organic carbon (TOC), chemical oxygen demand (COD), nitrite- and nitrate-nitrogen, orthophosphate, pH, and dissolved oxygen. All analytical procedures were conducted in accordance with Standard Methods (APHA, 1976). Dissolved oxygen measurements were taken on-site with a portable oxygen meter. A pH meter was used to measure pH of the samples in the laboratory. Temperature readings were taken at each sampling time. In addition, temperature was continuously monitored during a portion of each test run. This was accomplished by placing a calibrated thermistor at each sampling depth within the ponds. The thermistors were connected to a switching device which, in turn, was connected to a recorder. At approximately 10-minute intervals, the signal from a different thermistor served as input to the recorder. In this way, the temperature at each sampling point within a pond was monitored 16 times over a 24-hour period.

ALGAL ANALYSIS

Algae productivity in the model holding ponds was measured indirectly by the quantification of chlorophyll-a. After samples were brought to the laboratory, a 50 to 250 ml aliquot of each was filtered through a glass fiber filter. The filter was then placed in a 15 ml tissue grinder with approximately 5 ml of 90 percent acetone and macerated until the algal cells were disrupted and the pigments extracted. The contents of the tissue grinder were transferred to a graduated glass centrifuge tube, and the volume brought up to 10 ml with 90 percent acetone. This mixture was spun at high speed in a table-top clinical centrifuge for 5 minutes, after which the supernatant was decanted off and retained. This pigment/acetone solution was subjected to visible light spectral analysis at 662 and 750 nm on a spectrophotometer. The solution was then acidified with 0.1 ml of a 1.0 N HCl solution and its visible light absorbance at 662 and 750 nm was recorded. These four readings were then used in formulas given by Golterman (1971) for the quantification of chlorophyll-a in the original sample.

FIELD SAMPLING

As seasonal variation can have important effects on the effluent quality of a waste stabilization or holding pond, it was

necessary to monitor the fate of enteroviruses in the model ponds over a wide range of environmental conditions. Therefore, field tests were conducted during the months of the year best reflecting the seasons in Central Texas. Tests were begun in late October (Fall, 1975), mid-January (Winter, 1976), early March (Spring, 1976), mid-July (Summer, 1976) and mid-January (Winter, 1977).

The day before the initiation of a field test, wastewater was transported from the Govalle Wastewater Treatment Plant to the field site as previously described. The ponds were filled to within six inches of the top. Preliminary testing indicated that 24 hours was enough time to insure natural dechlorination of the wastewater. Therefore, this holding period, between filling the ponds with wastewater and adding virus to the ponds, was maintained throughout the field tests. After 24-hour interval virus inoculum for each pond, in 1-liter volumes, was transported under refrigeration to the site. The virus was added to the pond and then mixed for 15 minutes using an industrial mixer. The virus titer of the inoculum was such that levels of any indigenous viruses in the ponds were exceeded by 5-6 orders of magnitude. After mixing, the pond was allowed to become quiescent, and then the sediment samplers were lowered into the pond. Initial liquid sampling took place one hour after mixing. The sampling of the liquid then approximated the following schedule for each field test: 1 day, 3 days, 7 days, 10 days, 14 days, 17 days, 21 days, then weekly until no virus was detected in the water samples. Evaporation from the ponds was compensated for by the addition of distilled water to each pond after field sampling was done.

During the Spring and Summer of 1976, an attempt was made to simulate the dynamic flow conditions of six operational ponds. This was accomplished by removing approximately 33 percent of the liquid in selected model ponds and replacing it with an equivalent amount of fresh wastewater. Virus was added, the pond mixed, and sampling begun as has been described above.

LIQUID SAMPLING

Sampling of the liquid was done through rigid plastic tubes suspended in the pond. The tubes were mounted through holes drilled in a 2" x 4" x 5' board. This board was attached to the sides of the tank and spanned the pond two feet out from one side. Above this board the tubes were attached to flexible plastic tubing which extended over the edge of the pond. This tubing, in turn, was mounted through a hole in a rubber stopper that fit into the mouth of a liter sampling bottle. When samples were taken, a portable vacuum pump was connected to another hole in this stopper. A partial vacuum was formed in the sampling bottle and one liter of liquid was withdrawn from the pond. All pond samples were taken 1" below the liquid surface and 1" above the pond bottom. In the 90" deep ponds a third sample was taken about 45" beneath the pond surface. The samples were transported to the laboratory

immediately after collection and an aliquot was held at 4C until viral and coliform assays were performed. Microbial assays were conducted within 24 hours of sample collection.

SEDIMENT SAMPLING

Sediment samplers were made from cut-off plastic bleach bottles, six inches in diameter. Each bottle was weighted with about one-fourth inch of washed gravel and then suspended from three lengths of nylon cord. After virus was mixed into the pond for 15 minutes, 12 to 15 samplers were lowered into each pond.

Sediment was collected by removing the samplers from the pond and aspirating off all but 500 to 1000 ml of the overlying liquid. The sediment and remaining liquid were mixed into a slurry and poured into a sample bottle. The sampler was rinsed several times with distilled water to remove the remaining sediment. In the laboratory, the sediment was resuspended into the liquid and a portion of this mixture poured into a 200 ml centrifuge bottle. The sediment was separated from the liquid by centrifuging at a force of 1500 x g, turning off the power and allowing the rotor to coast to a stop. The overlying liquid was removed by aspiration. This procedure was repeated until all the sediment from a particular sample was collected in one centrifuge bottle. The pellet was then resuspended in 200 ml of phosphate buffered saline (PBS).

Viral assay was carried out either by direct plating of the sediment or by dispersing the solids and then centrifuging and plating the supernatant. When direct plating was used, the solids were removed from the cells by washing with 5 ml of PBS containing 500 units/ml of penicillin and 250 µg/ml streptomycin after the 45-minute infection period. Sonication was used as a means of dispersing or disrupting the sediment solids (in PBS) and releasing the virions. The sediments were sonicated in 50 ml plastic centrifuge tubes placed in an ice-water bath using a Branson laboratory sonicator set at six amperes. After 3 minutes of sonication, the sample was spun at 12,000 x g for 10 minutes and the supernatant was assayed for virus. This procedure was particularly effective when virus titers in the sediments were low.

LABORATORY STUDIES

Temperature Studies

One liter of final effluent was dechlorinated with five ml of 1 N sodium thiosulfate. To simulate primary effluent, raw wastewater was allowed to settle for one hour; after this period, one liter of the supernatant was decanted for experimental use. Both wastewaters were then divided into three-300 ml aliquots; poliovirus 1 (Chat) was seeded into each aliquot at a final concentration of approximately 1 x 10^6 pfu/ml; and a test volume of each suspending medium was placed at 4C, 20C, and 30C. These were

sampled periodically for the presence of poliovirus. Additionally, chemical analyses performed on the wastewaters included suspended solids, total organic carbon, pH, and specific conductivity.

Similar experiments were performed with Coxsackievirus B-3 (Nancy). However, in this case wastewater was divided into three-200 ml aliquots and seeded with Coxsackievirus at a final concentration of about 1×10^6 pfu/ml. The three aliquots of each suspending medium were then placed at the test temperatures sampled for viral infectivity.

Light Studies

Poliovirus was added to either primary or secondary dechlorinated effluent to a final concentration of approximately 5×10^4 pfu/ml. Duplicate aliquots of each seeded effluent were placed in 4C and 20C environmental rooms under continuous overhead fluorescent lighting of 310 foot-candles at a distance of 15 inches. An identical set of duplicate samples was covered with foil to exclude light and was placed in the same environmental rooms. Poliovirus infectivity as pfu was monitored with time.

Dissolved Oxygen Studies

Final and primary effluent samples were brought to the laboratory and dechlorinated as required. Four liter volumes of each type of effluent were placed in 4C and 20C environmental rooms. The following day each volume was seeded to a final concentration of approximately 5×10^4 pfu/ml poliovirus. After mixing, the volumes were divided into four-1 liter aliquots. Two of these were sealed and two were kept under constant aeration using air-stone diffusion. All samples were assayed periodically for poliovirus.

Hydrogen Ion Concentration Studies

Buffer stock solutions were made by dissolving the proper proportions of inorganic reagents (Dobos, 1975) in dechlorinated final effluent. These stocks were stored at 4C until used. Buffer mixtures were made by adding the correct proportions of stock solution to dechlorinated final effluent. These 200 ml volumes were brought to 20C in an environmental room, and either poliovirus or Coxsackievirus was added to each mixture at a final concentration of approximately 1×10^4 pfu/ml. The sample volumes were held quiescent at 20C and monitored for pH and the presence of infectious virus.

Algae Studies

An unknown species of non-filamentous green algae was isolated from final effluent. The treated wastewater was sprayed in a fine mist onto 100 mm petri dishes containing 20 ml Bold Basal algae culture medium (Nichols, 1973) in 1.5% agar. The plates were incubated in an algae culture room under continuous lighting at 20C

until colonies were large enough to be seen. Individual colonies were transferred aseptically to flasks containing 100 ml of sterilized Bold Basal Medium. After sufficient incubation these cultures were used to inoculate the wastewater used in the experiment.

One week prior to the experiment, fresh final effluent was brought to the laboratory. One portion was held at 4C in an environmental room. A second portion was filtered through an asbestos filter sheet (Hercules Sterilizing Filter Sheet, grade ST3) to remove particulates and bacteria. One liter each of filtered and unfiltered effluent were then seeded with 20 ml of the algae suspension and incubated. The remaining filtered effluent was held in the environmental room at 4C. The day before the experiment, the two flasks containing the algae cultures were placed at 4C along with the unseeded flasks.

On the day of the experiment, flasks containing one liter of either unfiltered or filtered final effluent, and one liter of unfiltered or filtered final effluent plus algae were placed on magnetic stirrers in the 4C environmental chamber. Poliovirus was added to each volume at a final concentration of about 1×10^2 pfu/ml. Ten ml samples were taken and filtered through a 0.45 µm membrane filter 47 mm in diameter. The solids retained on the filter were washed off using 10 ml of tryptose phosphate broth in a syringe equipped with an 18 gauge needle. The filtrate and filter surface wash were assayed for poliovirus.

There was a possibility that virus might be retained on the mat of algae formed on the surface of the membrane filter. To determine the amount of retention on such a mat, three 10 ml aliquots of the final effluent-algal culture were filtered through three separate membrane filters before any virus had beed added. Virus then was added to clarified final effluent, and a 10 ml sample was filtered through each of the above filter mats. The filtrate and tryptose phosphate wash of these filters were assayed for poliovirus.

It was of interest to see if elution media other than tryptose phosphate broth might be more effective in washing virus off the membrane filters. To this end, at the 1 hour and 24 hour sampling times triplicate 10 ml samples were taken from the final effluent-algae culture and the clarified effluent-algae culture. These were filtered and the filters washed with 10 ml of either tryptose phosphate broth, glycine-EDTA buffer (pH9), or distilled water. Five ml of this wash was removed for direct plating and the remainder centrifuged at 12,000 x g for 10 minutes to remove the solids. The supernatant was retained for viral assay.

Because pH appeared to have a major effect on the rate of virus inactivation, an experiment was designed to evaluate the effects of naturally high and low pH levels on poliovirus in the presence (or absence) of algae. Four 1 liter aliquots of primary effluent and dechlorinated final effluents were measured into

Erlenmyer flasks. Two liters of each effluent were inoculated with 10 ml of a single-cell algae culture and placed in a 20C environmental room under continuous lighting. The other aliquots were covered to exclude light, sealed and stirred slowly on magnetic stirrers in that same room. All test aliquots were monitored until conditions of high pH and dissolved oxygen were reached in the algae seeded volumes and low pH and dissolved oxygen were present in the sealed volumes. Approximately 5 x 10^4 pfu/ml poliovirus were added to each volume. Each system was then monitored for viral infectivity.

STATISTICAL ANALYSIS

In the process of the statistical analysis, the following questions were addressed:

1. Does poliovirus survival differ between sampling points within a pond?
2. What is the relationship between poliovirus survival and such variables as the holding period (time points of observation), pH, and temperature?
3. Does the survival model differ from pond to pond within seasons?
4. Does the model differ from season to season within each pond?
5. What is the effect of final or primary effluent on poliovirus survival?

For each pond during each field test models of virus survival incorporating detention time, pH, and temperature as explanatory variables were developed using a multiple linear regression program (BMDPIR).

The models used were:

$$\ln (y + 1) = \alpha_0 + \alpha_1 p + \alpha_2 t + \varepsilon \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

and

$$\ln (y + 1) = \alpha_0 + \alpha_1 p + \alpha_2 t + d_3 T + \varepsilon \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

where y is the number of recoverable virions, p is the pH and T is the temperature (^oC) at time t. The uncontrolled error term, ε, represents the total effect not accounted for by the explanatory variables.

Since no single model was adequate for all of the conditions, a comparison of the models was necessary to determine whether some models were adequate for describing the poliovirus survival under

selected conditions. There comparisons were made using a program of analysis of variance and covariance (BMDPIV) from the same package. This program permits overall comparisons among all models as well as pairwise comparisons between models. It is worth mentioning that analysis of a linear model which involves both the quantitative as well as qualitative variable is called analysis of covariance. Analysis of a model involving only qualitative variables is called analysis of variance, whereas, analysis of a model involving only quantitative variables is called regression analysis. In this study, comparing models under different conditions involved both types of variables (quantitative as well as qualitative), thus analysis of covariance was performed. Before the results of these comparisons can be discussed, the statistical quantities used in evaluating the data must be explained.

A major portion of the statistical evaluation used in this study relies on the use of three terms: R^2, Standard Error and Model. The quantity R^2 represents the extent to which the associated model fits the data, 100 R^2 represents the percent variation explained by the model or by the explanatory variables in the model, and 100 $(1-R^2)$ is the percent variation unaccounted for by the explanatory variables in the model. Standard error gives an estimate of variability in the data. For a good model the standard error should be small. If a new variable is introduced into a model and the standard error becomes greater than that of the previous model, then this indicates that the inclusion of the new variable is not meaningful.

The significance of individual coefficients in the models can be determined by the value of an associated probability, usually printed on the computer output. If this probability value is greater than some preassigned probability (α) called the level of significance, then the coefficient is not significant. If this probability is less than α, then the coefficient is significant. The level of significance used for this study was 0.10.

Two hypotheses were tested regarding these models: equality of intercepts and equality of slopes. These tests involve three statistical quantities: the degrees of freedom, the observed F value and the probability value (P) associated with this F statistic. P is compared with the level of significance (α = .10). If for a given comparison P > .10 either for the test of equality of intercepts or equality of slopes, then the corresponding hypothesis is rejected. The degrees of freedom are constants associated with F which depend on the sample size and the hypothesis tested.

RESULTS AND DISCUSSION

LABORATORY STUDIES

Controlled Temperature Studies

Chemical and physical analyses of the suspending media are presented in TABLE 10. The inactivation of poliovirus and Coxsackievirus in the different wastewaters at the three indicated temperatures is presented graphically in FIGURES 11 and 12 where percent of recoverable virus is graphed as a function of time. It is clear that there is prolonged survival of both poliovirus and Coxsackievirus at lower temperatures (e.g., after 5 days in final effluent there is 98, 4 and 1% reductions of poliovirus at 30C, 20C and 4C, respectively).

TABLE 10. RESULTS OF CHEMICAL ANALYSIS OF WASTEWATERS USED IN THE TEMPERATURE STUDIES.

Parameter	Final Effluent	Primary Effluent
	Poliovirus I (Chat)	
Total Suspended Solids (mg/l)	13.2	112.
Total Volatile Suspended Solids (mg/l)	1.6	88.
Total Organic Carbon (mg/l)	24.	130.
pH (units)	7.6	7.3
Specific Conductivity (μmho/cm^2)	870.	770.
	Coxsackievirus B-3	
Total Suspended Solids (mg/l)	17.	49.0
Total Volatile Suspended Solids (mg/l)	9.	27.2
Total Organic Carbon (mg/l)	19.	--
pH (units)	7.6	--
Specific Conductivity (μmho/cm^2)	805.	--

FIGURE 11. THE SURVIVAL OF POLIOVIRUS (CHAT) AT 4, 20 AND 30C.

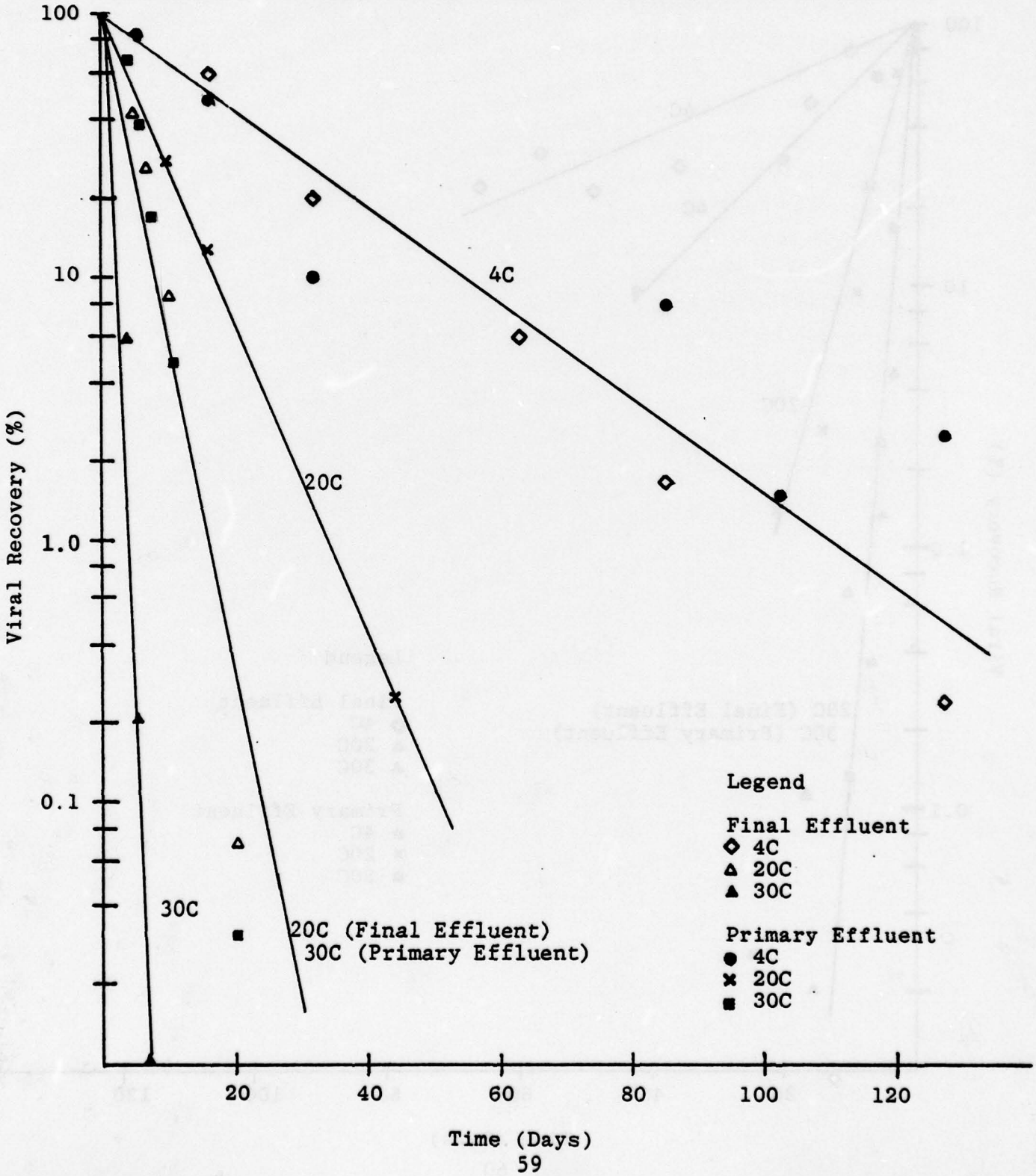
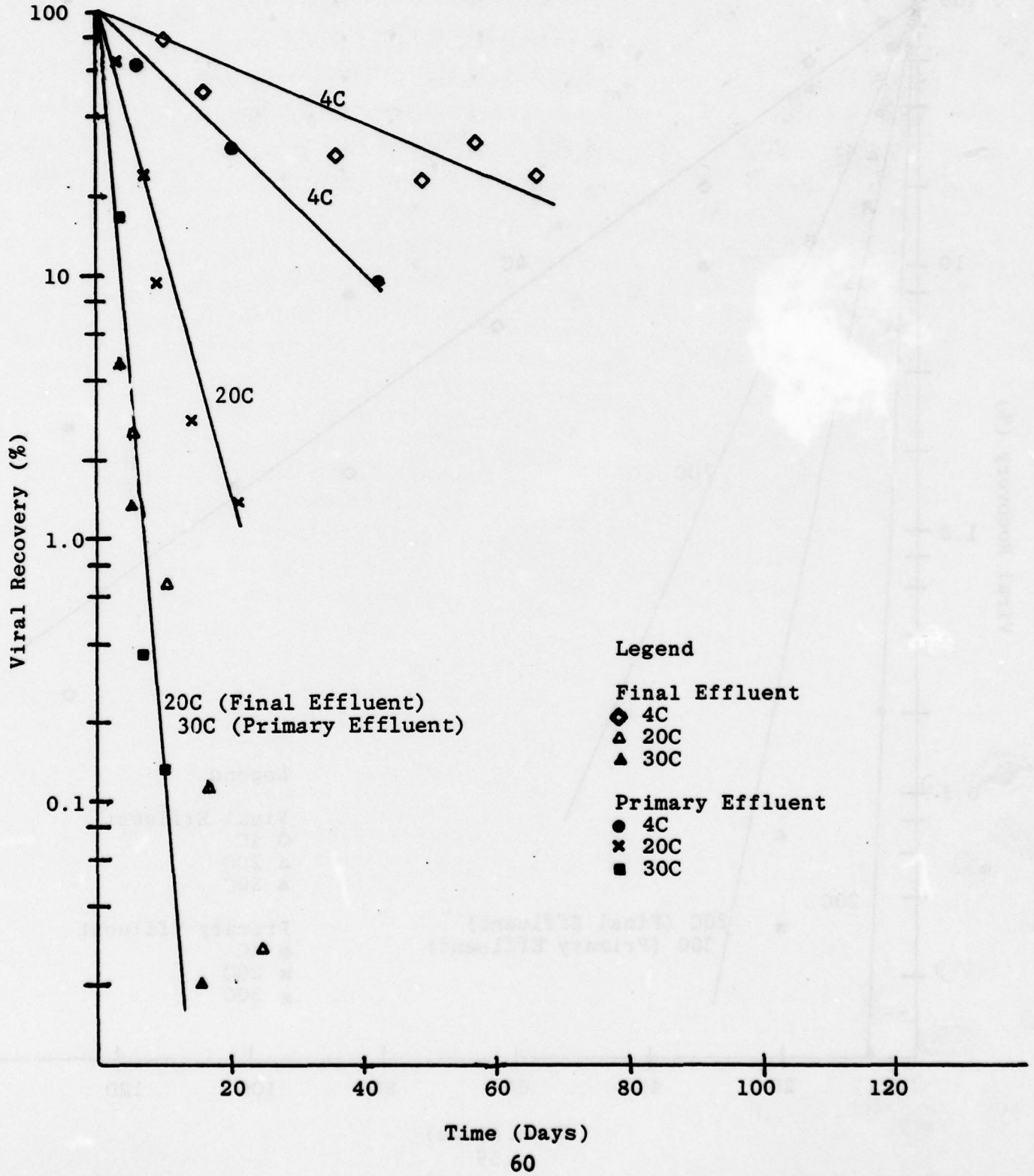


FIGURE 12. THE SURVIVAL OF COXSACKIEVIRUS B3 AT 4, 20 and 30C.



An examination of these data indicates that there is little difference in the rates of inactivation of the two viruses at 20 and 30C. However, there appears to be a significant difference in the inactivation rates of poliovirus and Coxsackievirus at 4C (i.e., a 94% vs. an 80% reduction in virus numbers after 60 days, respectively). Unfortunately, it is difficult to generalize on the relative resistance of the two viruses to inactivation on the basis of these data. It was not possible to conduct both experiments simultaneously; therefore, the wastewaters, although comparable, are not identical.

Further examination of the data shows that for each particular virus, survival is prolonged in settled sewage or primary effluent as compared to the final effluent at all temperatures tested. The reasons for this cannot be ascertained from these experiments, but the suggestion that solids protect viruses from inactivation (Schaub and Sagik, 1975) may help explain the survival differences observed here. Another possible explanation is the presence of increased organics in the primary effluent.

Light Studies

Results (as duplicate averages) for all test conditions are presented graphically in FIGURES 13 and 14. Additional physical parameters for each system are presented in TABLE 11. At 20C, differences in viral survival between light and dark conditions in final effluent are indistinguishable. However, in the other experimental systems, poliovirus recoverability was greater under dark conditions. It was shown in the controlled temperature studies that the survival of poliovirus is prolonged at lower temperatures and in primary effluent. At 20C the inactivation of poliovirus in final effluent is so rapid that the effects of light on virus survival cannot be distinguished from other effects. However, the protection afforded the virus by suspension in primary effluent at 20C and in final effluent at 4C makes it possible to observe the antiviral effects of light on poliovirus. The results reported here are in agreement with those of Bitton (personal communication) who has studied the inactivation of poliovirus by sunlight.

Dissolved Oxygen Studies

Virus survival under the conditions previously described is illustrated in FIGURES 15 and 16. Additional physical parameters are given in TABLE 12. Survival of poliovirus at 4C is longer for both effluents and for both the aerated and sealed test volumes than it is at 20C. In final effluent, at both temperatures, the aerated volumes appear to have a slightly lower rate of poliovirus inactivation than do the sealed volumes. However, the absence of aeration does not significantly affect virus survival at either temperature. In primary effluent the trends of inactivation are reversed, i.e., poliovirus in the sealed volumes have a somewhat prolonged survival over those in

FIGURE 13. POLIOVIRUS SURVIVAL UNDER CONDITIONS OF LIGHT AND DARK IN FINAL EFFLUENT.

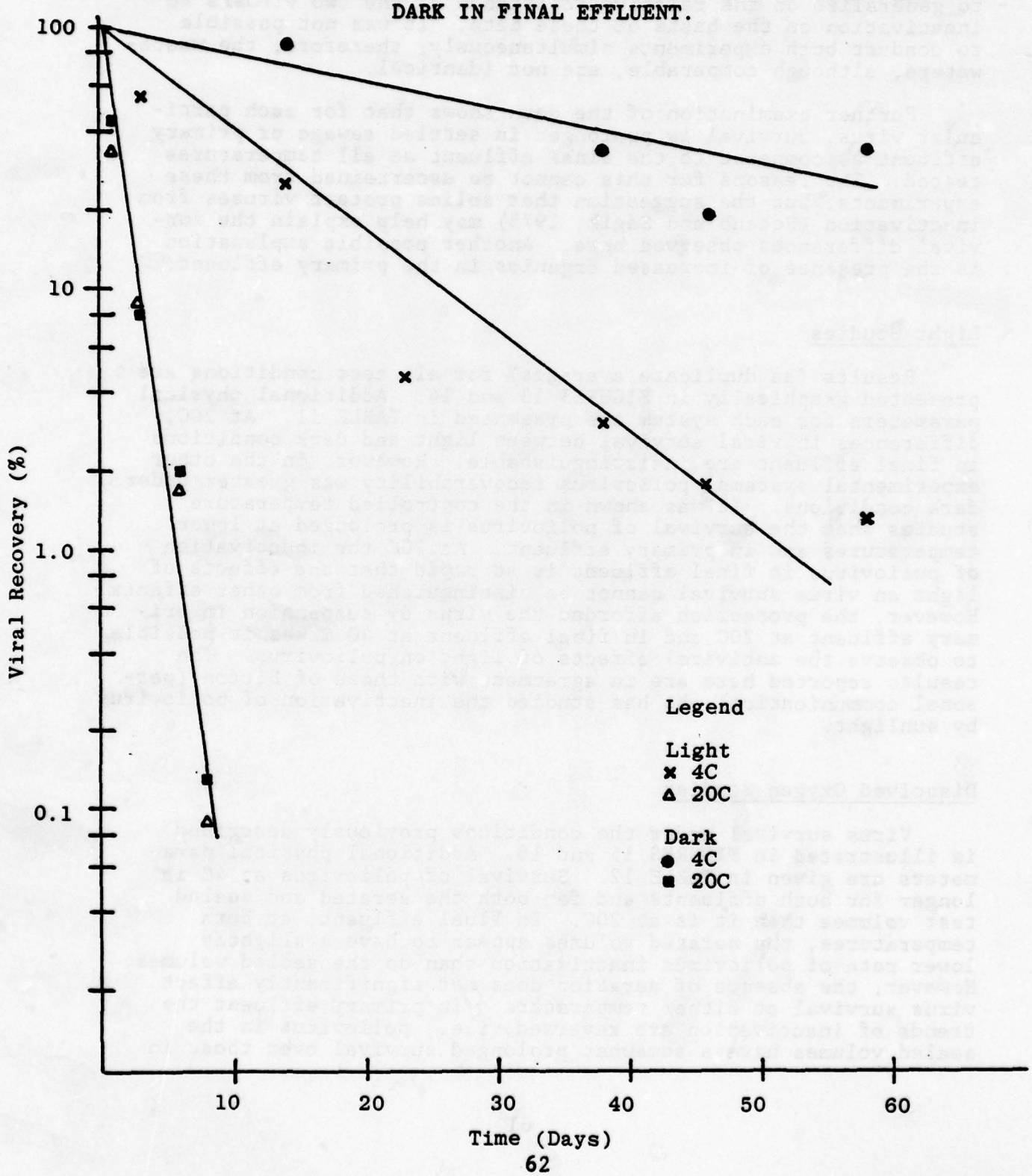


FIGURE 14. POLIOVIRUS SURVIVAL UNDER CONDITIONS OF LIGHT AND DARK IN PRIMARY EFFLUENT.

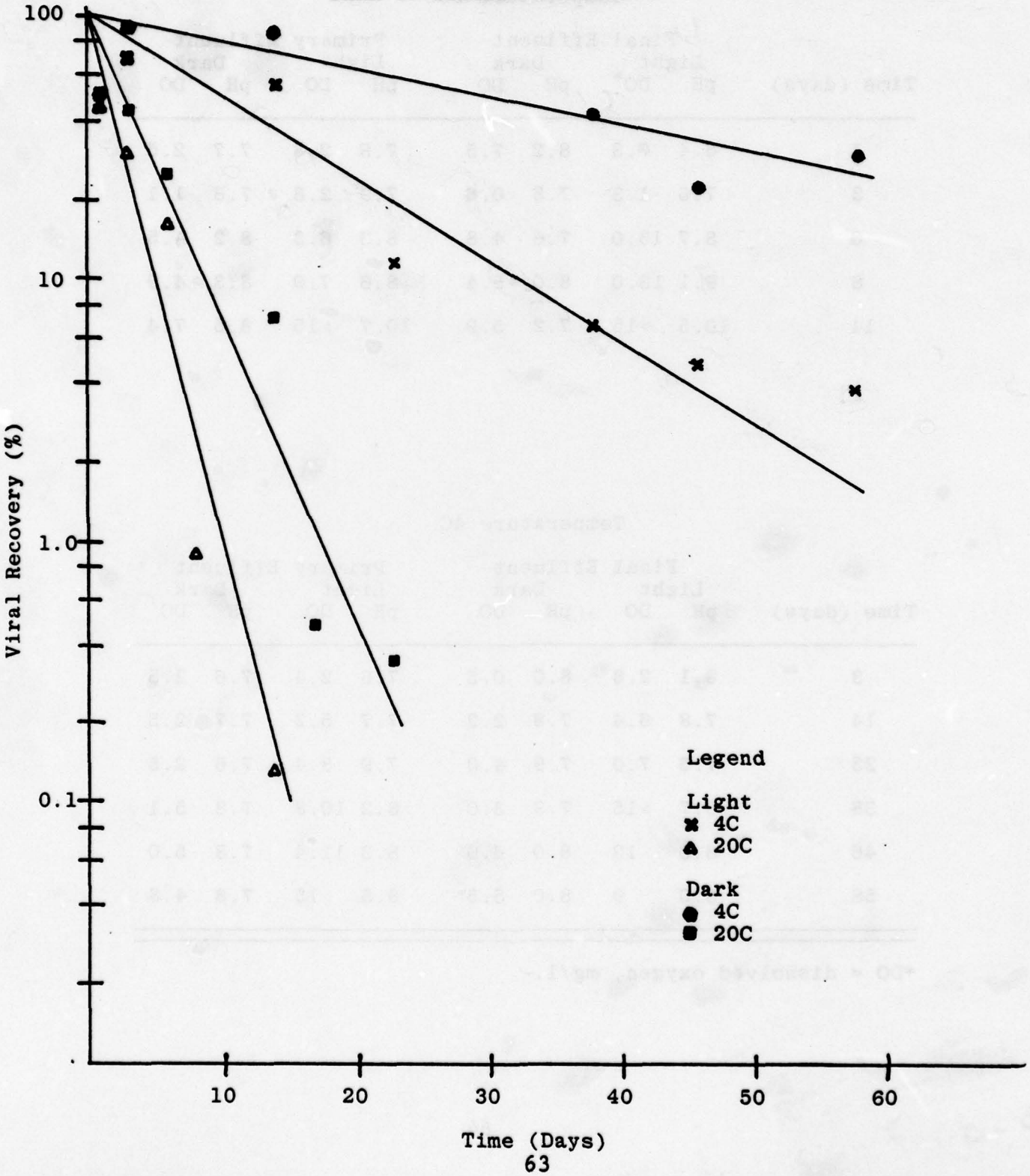


TABLE 11. pH AND DISSOLVED OXYGEN CONCENTRATIONS OF TEST VOLUMES IN LABORATORY LIGHT STUDIES.

Temperature 20C

Time (days)	Final Effluent Light pH	Final Effluent Light DO*	Final Effluent Dark pH	Final Effluent Dark DO	Primary Effluent Light pH	Primary Effluent Light DO	Primary Effluent Dark pH	Primary Effluent Dark DO
1	8.4	6.8	8.2	7.5	7.8	2.8	7.7	2.5
3	7.6	1.3	7.8	0.6	7.9	2.8	7.8	1.1
6	8.7	13.0	7.6	4.8	8.3	6.3	8.2	4.5
8	9.1	13.0	8.0	5.4	8.6	7.9	8.3	4.9
14	10.5	>15	7.2	5.9	10.7	>15	8.5	7.4

Temperature 4C

Time (days)	Final Effluent Light pH	Final Effluent Light DO	Final Effluent Dark pH	Final Effluent Dark DO	Primary Effluent Light pH	Primary Effluent Light DO	Primary Effluent Dark pH	Primary Effluent Dark DO
3	8.1	2.8	8.0	0.5	7.6	2.4	7.6	2.5
14	7.8	6.4	7.8	2.2	7.7	5.2	7.7	2.5
23	7.6	7.0	7.9	4.0	7.9	8.4	7.6	2.5
38	8.7	>15	7.9	3.0	8.2	10.8	7.8	5.1
46	8.6	12	8.0	6.0	8.3	11.4	7.8	5.0
58	8.0	9	8.0	5.5	8.5	>15	7.8	4.8

*DO = dissolved oxygen, mg/l.

FIGURE 15. POLIOVIRUS SURVIVAL IN THE PRESENCE AND ABSENCE OF DISSOLVED OXYGEN IN FINAL EFFLUENT.

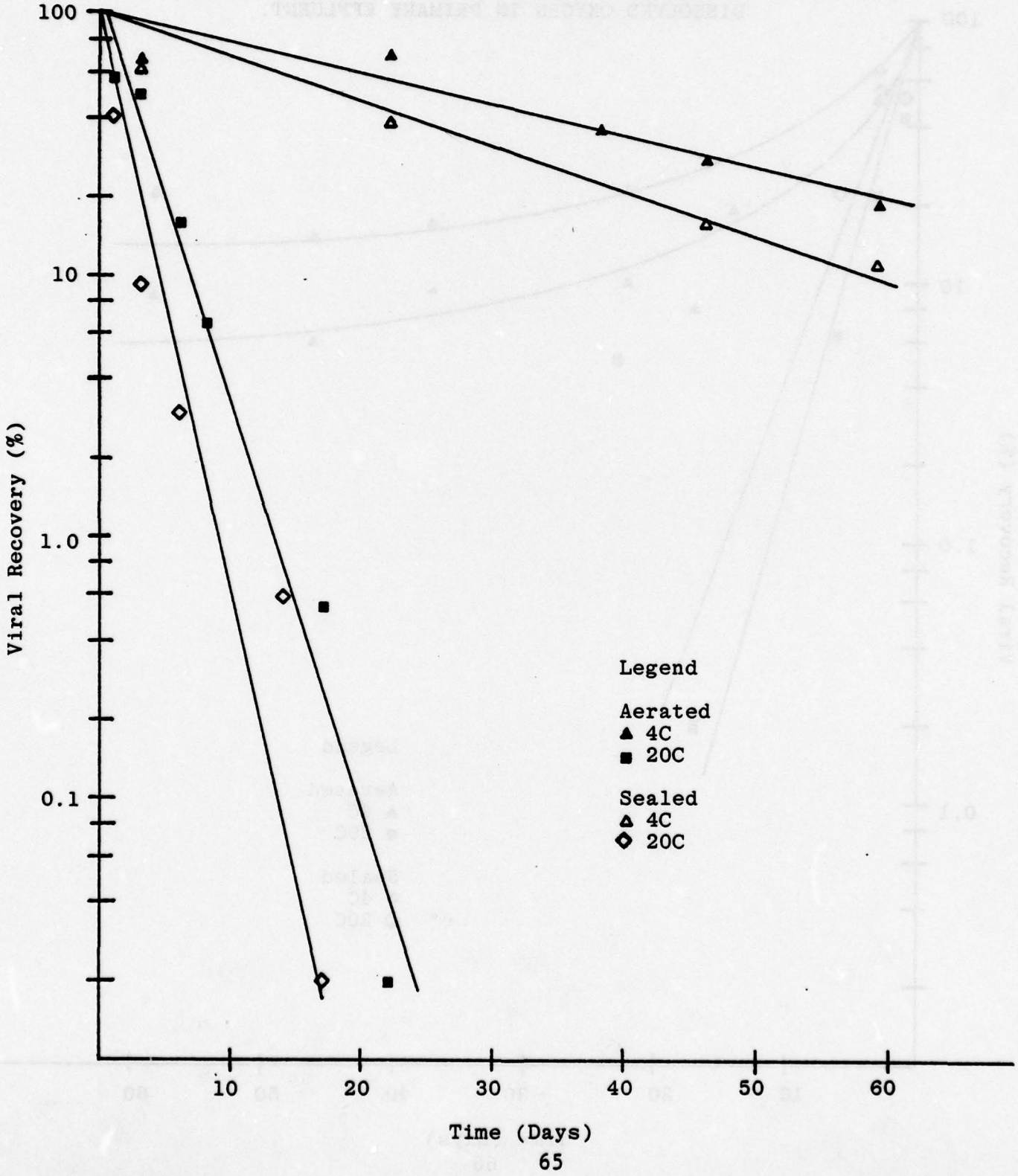


FIGURE 16. POLIOVIRUS SURVIVAL IN THE PRESENCE AND ABSENCE OF DISSOLVED OXYGEN IN PRIMARY EFFLUENT.

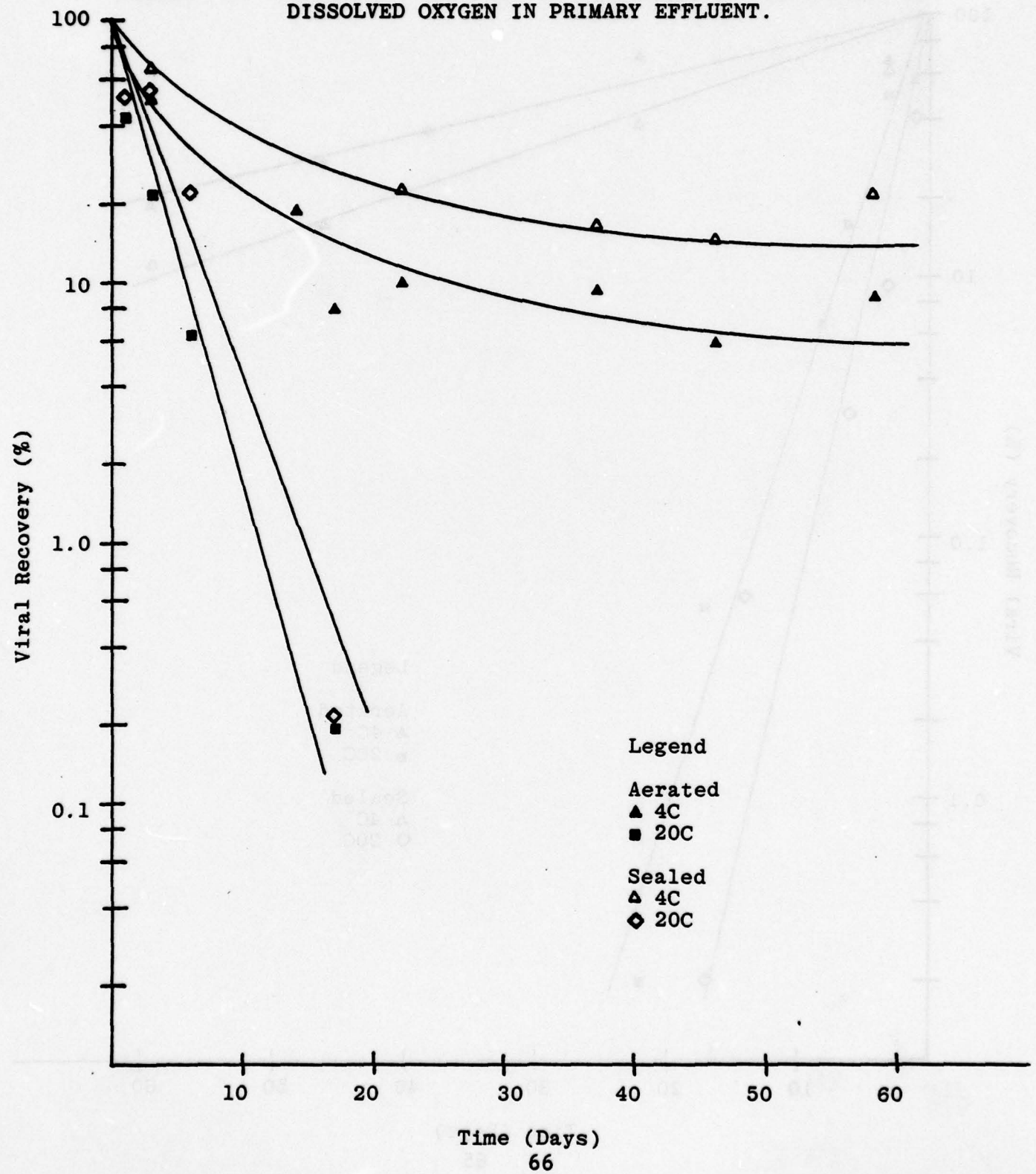


TABLE 12. PH AND DISSOLVED OXYGEN CONCENTRATIONS OF TEST VOLUMES IN DISSOLVED OXYGEN LABORATORY STUDY.

Temperature 20C

Time (days)	Final Effluent plus air DO*	pH	Final Effluent Sealed DO	pH	Primary Effl. plus air DO	pH	Primary Effl. Sealed DO	pH
1	7.6	8.5	6.8	7.9	7.5	8.7	3.0	7.5
3	8.6	8.5	0.9	7.1	8.4	8.6	1.0	7.3
6	8.2	8.2	0.8	7.1	8.4	8.7	1.0	7.3
8	7.6	8.4	1.6	6.8	7.6	8.7	2.0	7.2
14	8.8	8.3	4.0	6.9	7.0	8.7	3.2	7.2
17	7.7	7.9	4.0	6.5	7.5	8.5	3.4	7.2
23	8.2	7.8	3.0	7.5	8.2	8.5	4.0	7.1

Temperature 4C

Time (days)	Final Effluent plus air DO	pH	Final Effluent Sealed DO	pH	Primary Effl. plus air DO	pH	Primary Effl. Sealed DO	pH
3	12	8.5	6	7.9	12	8.5	2.2	7.4
8	12	8.4	3	7.8	9	8.5	1.0	7.3
14	13	8.4	7	8.0	13	8.4	1.0	7.2
17	13	8.4	6.5	7.9	12	8.4	1.0	7.1
23	13	8.6	7	7.8	13	8.4	1.0	7.2
38	12	8.5	3.5	7.7	12	8.4	4.0	7.2
46	12	8.3	3.5	7.6	12	8.4	3.0	7.4
58	12	8.3	5	7.6	12	8.4	7.0	7.2

* Dissolved Oxygen (mg/l)

the aerated volumes. Again the presence or absence of air does not appear to alter the rate of poliovirus inactivation significantly.

Hydrogen Ion Concentration Studies

The results of the experiments using poliovirus and Coxsackievirus respectively, are presented graphically in FIGURES 17 and 18. On these graphs each line is a profile of virus survival in final effluent from pH 5.0-10.0 on the particular day indicated. The figures show the trend of poliovirus inactivation at 7, 11, 17 and 22 days after virus addition, and the trend of Coxsackievirus inactivation 12, 30 and 34 days after addition. It is apparent that both viruses are most stable around pH 7.0. Stability falls off on either side of neutrality.

The results of these experiments are interesting in the light of what is observed in operating ponds. If pH is the sole criterion considered, one would expect higher rates of virus inactivation in the region of active photosynthesis - that is, near the pond surface rather than below the euphotic zone. It would also be expected that virus survival would be prolonged near the bottom of the pond, as anaerobic conditions here would hold the pH near neutrality. Virus survival during the winter may also be prolonged as phytoplankton activity is often diminished and, therefore, pH levels will be reduced in these months.

Algae Studies

The results of the interactions of poliovirus with effluent solids and algae are presented in TABLE 13. There appears to be association of poliovirus with particulates in both the unfiltered final effluent and the unfiltered effluent containing algae. In contract, low numbers of virus were recovered from the filters through which filtered final effluent or filtered effluent containing algae had been passed. This indicates that while there is association of poliovirus with wastewater particulates, there is little association of the virions with algal solids.

The results (as duplicate averages) of adding poliovirus to algal-wastewater systems with naturally high or low levels of hydrogen ion concentration are presented in FIGURE 19. Here poliovirus survival is graphed as a percent of those virions recoverable at one day. Additional parameters are presented in TABLE 14. These data reveal that the rate of virus inactivation in the algae cultures is much greater than in the anaerobic systems. The differences in the inactivation rate cannot be ascribed directly to pH, as the microbial community of the systems differed. However, as the pH of the algae cultures was always greater than 9.0 while that of the sealed volumes was near neutrality, it is evident that the pH differences induced by the different predominant microorganisms may well have contributed to differential rates of poliovirus inactivation. Dissolved oxygen levels were

FIGURE 17. POLIOVIRUS INACTIVATION IN FINAL EFFLUENT AS A FUNCTION OF pH.

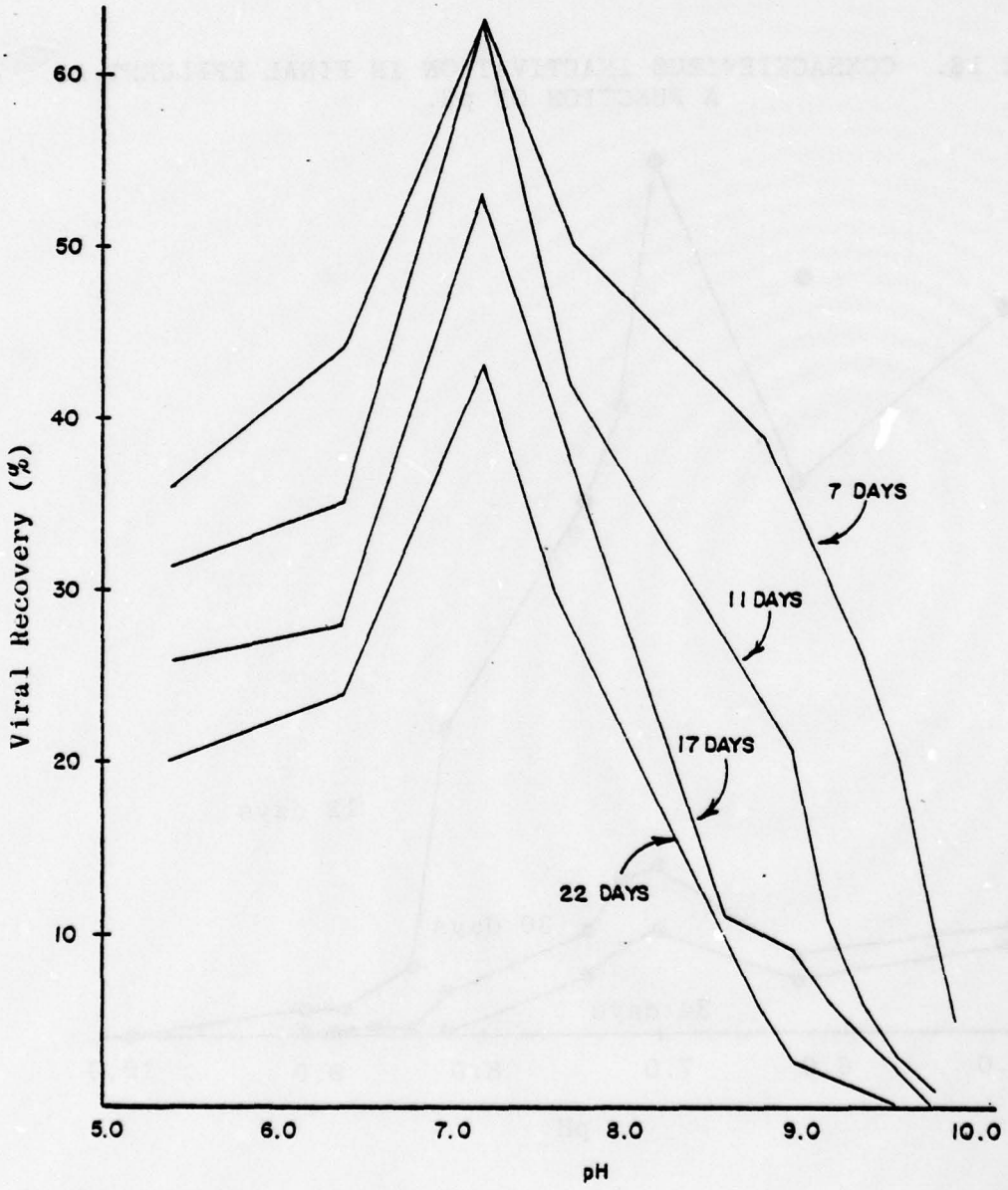


FIGURE 18. COXSACKIEVIRUS INACTIVATION IN FINAL EFFLUENT AS A FUNCTION OF pH.

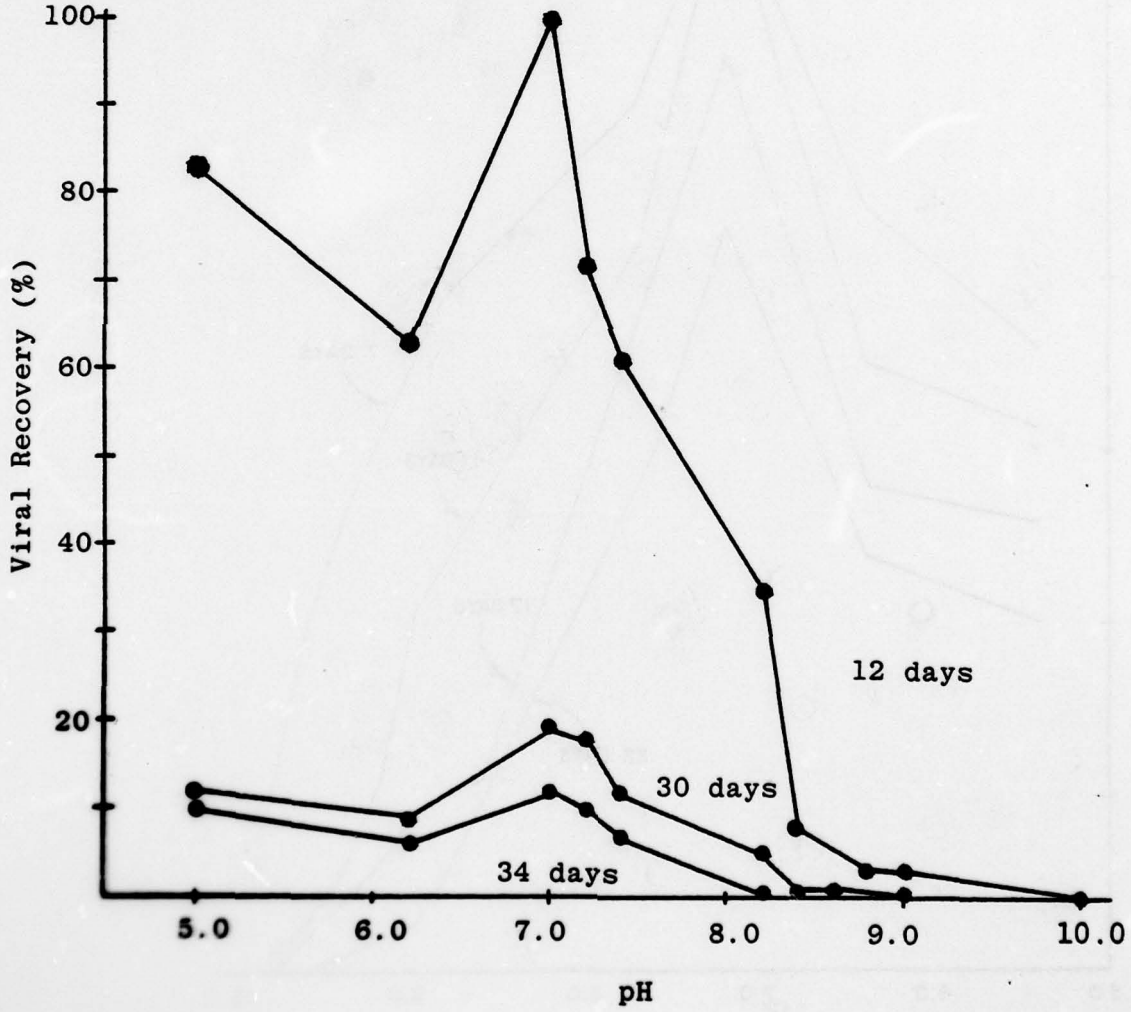


TABLE 13. ASSOCIATION OF VIRUS WITH FINAL EFFLUENT SOLIDS AND ALGAE.

Time	Unfiltered Effluent		Filtered Effluent	
	% Virus in Filtrate[α]	% Virus Recovered from Filter[α]	% Virus in Filtrate	% Virus Recovered from Filter[α]
0	88.8	11.2	99.00	0.99
5"	83.08	16.92	99.49	0.51
15"	99.58	0.42	99.75	0.25
30"	97.63	2.37	100	0
1'	97.8	2.2	99.53	0.47
4'	97.56	2.44	99.78	0.22
24'	97.88	2.12	99.73	0.23

Time	Unfiltered Effluent plus Algae[ε]		Filtered Effluent plus Algae[ρ]	
	% Virus in Filtrate[α]	% Virus Recovered from Filter[α]	% Virus in Filtrate[α]	% Virus Recovered from Filter[α]
0	97.91	2.08	100	0
5"	99.51	.49	99.49	0.51
15"	97.46	2.54	99.27	0.73
30"	99.77	3.23	99.53	0.47
1'	95.13	4.87	99.03	0.97
4'	92.11	7.9	99.48	0.52
24'	87.76	12.24	100	0

Algae Mat Control

% Virus in Filtrate	% Virus Recovered from Filter
99.51[σ]	0.49[σ]

α These figures are based on the total pfu detected in the sample at each particular sampling time.

ε 2638 µg/l chlorophyll

ρ 1627 µg/l chlorophyll

σ Figures are averages of triplicate samples

FIGURE 19. POLIOVIRUS SURVIVAL IN ALGAE-WASTEWATER SYSTEMS WITH NATURALLY LOW OR HIGH pH.

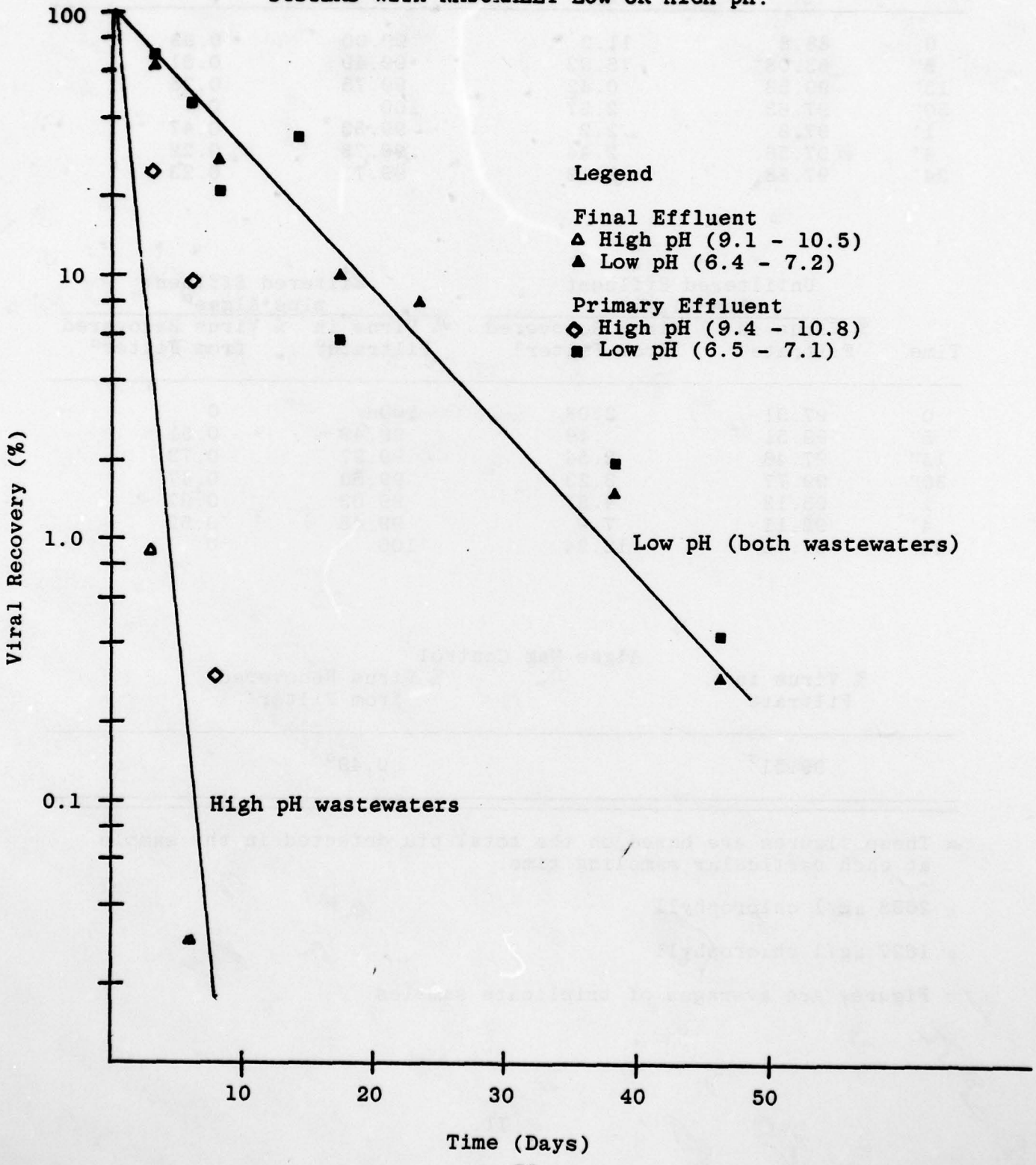


TABLE 14. PH AND DISSOLVED OXYGEN LEVELS IN FINAL AND PRIMARY EFFLUENT CONTAINING ALGAE AND IN SEALED EFFLUENT VOLUMES.

Time (days)	Final Effluent plus Algae		Final Effluent Sealed	
	pH[α]	DO[αε]	pH	DO
0	9.1	11.5	7.0	6.1
1	9.5	11.0	7.1	4.5
3	10.9	>15.0	6.9	0.5
6	10.8	>15.0	6.9	0.4
8	10.5	9.6	6.8	0.7
10			6.8	0.5
14			6.7	0.6
17			6.8	0.5
23			6.7	1.3
38			6.7	3.0
46			6.5	1.6

Time (days)	Primary Effluent plus Algae		Primary Effluent Sealed	
	pH	DO	pH	DO
0	9.5	11.3	7.2	5.7
1	9.9	12.5	7.2	4.5
3	10.3	>15.0	6.9	0.5
6	10.8	>15.0	6.7	0.5
8	10.8	13.6	7.1	0.6
10			6.9	0.5
14			7.1	1.5
17			7.1	1.7
23			6.9	1.2
38			6.8	2.5
46			6.6	1.1

α Results presented as duplicate averages.

ε Dissolved Oxygen (mg/l).

always much higher in the algae cultures, but as has been indicated above, the presence or absence of dissolved oxygen does not seem to affect poliovirus survival per se.

FIELD STUDIES

Preliminary Field Tests

Early in the study (Fall 1975) one pond of each depth was filled with final effluent. The primary purpose of this test was to evaluate the sampling procedures and the monitoring equipment. A seven-day period elapsed between the introduction of the wastewater and the viral inoculation of the shallower ponds. During this period a large algae population became established in three of the four ponds. Consequently, environmental conditions in the shallow ponds were considerably different from those in the deep pond. One important difference was pH. It is clear from an examination of TABLE 15 that algae growth and metabolism resulted in a highly alkaline pH in the 18-, 30-, and 42-inch ponds.. The 90-inch pond experienced no such change in pH as algae did not become established during the test period.

FIGURE 20 reports the percent of recoverable viruses after addition as a function of time. Loss from the liquid portion of the ponds was quite rapid, approaching four orders of magnitude within 10 days in the shallow ponds. Loss in the deep pond was slower, reflecting, perhaps, the lower pH. Mean pond temperatures at 1000 hours were near 20C (TABLE 16) late afternoon temperatures were probably higher. It has been shown that 99% inactivation of poliovirus in final effluent can take place within 20 days at 20C in the laboratory. Therefore, it is probable that pond temperature was a significant factor in the rapid reduction of poliovirus in the ponds during this test. It was during the preliminary field tests that the inadequacy of the plastic as a pond liner was discovered. Consequently, the ponds were drained, dried and a new sealant applied and cured.

Winter Test Series, 1976

A field test using eight of the ponds filled with final effluent was begun in January. Two ponds were tested at each depth with Series 1 and Series 3 each consisting of identical ponds at 18-, 30-, 40- and 90-inches. Observation of virus removal and chemical and physical analysis were conducted during the entire test period.

The temperatures observed in the 18-, 30-, 42- and 90-inch ponds can be found in FIGURE 21. It can be seen that in the shallow pond little difference was observed between top and bottom: both points reflect immediate changes in ambient temperatures. In contrast, differences as great as 9C were observed between the top and the bottom of the 90-inch pond. The temperature at the bottom of this pond fluctuated little and did not

TABLE 15. VALUES OF pH IN FINAL EFFLUENT PONDS, FALL, 1975.

Time (days)	18"	30"	42"	90"
0	10.2	10.1	9.7	8.0
1	10.3	10.2	9.9	6.3
2	10.5	10.4	10.0	7.4
3	10.8	10.9	10.5	7.4
4				
5				7.5
6	10.5	10.5	9.8	
7				7.8
8	10.3	10.5	9.7	
9				
10	10.1	10.3	9.6	

TABLE 16. MEAN TEMPERATURES OF MODEL PONDS, FALL, 1975.

Pond	Depth	Temperature (^{o}C)
18"	Top	19
	Bottom	19
30"	Top	19
	Bottom	18
42"	Top	20
	Bottom	18
90"	Top	20
	Middle	21
	Bottom	21

FIGURE 20. POLIOVIRUS SURVIVAL IN FINAL EFFLUENT PONDS-FALL, 1975.

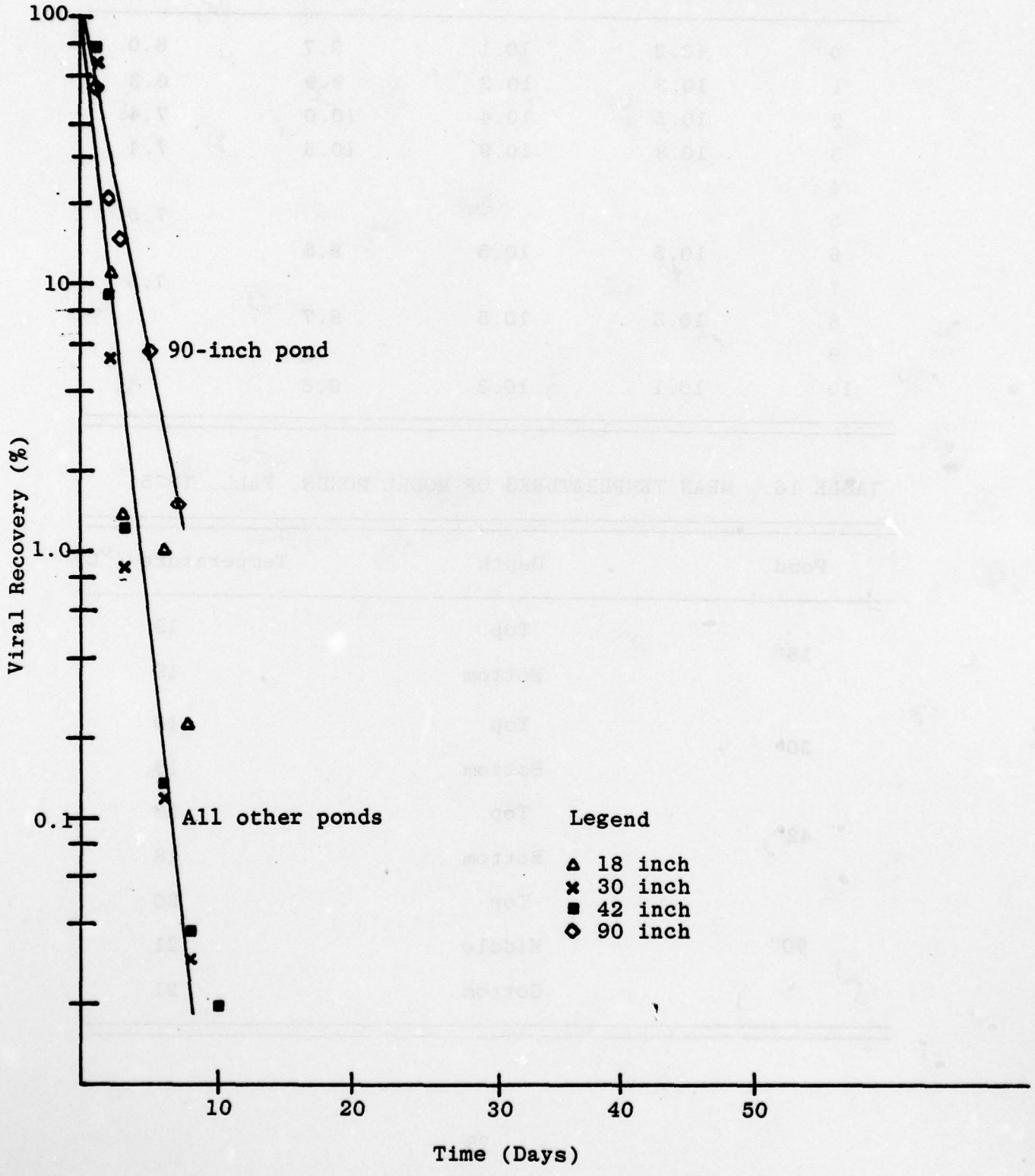


FIGURE 21. TEMPERATURE PROFILES OF MODEL HOLDING PONDS-WINTER, 1976.

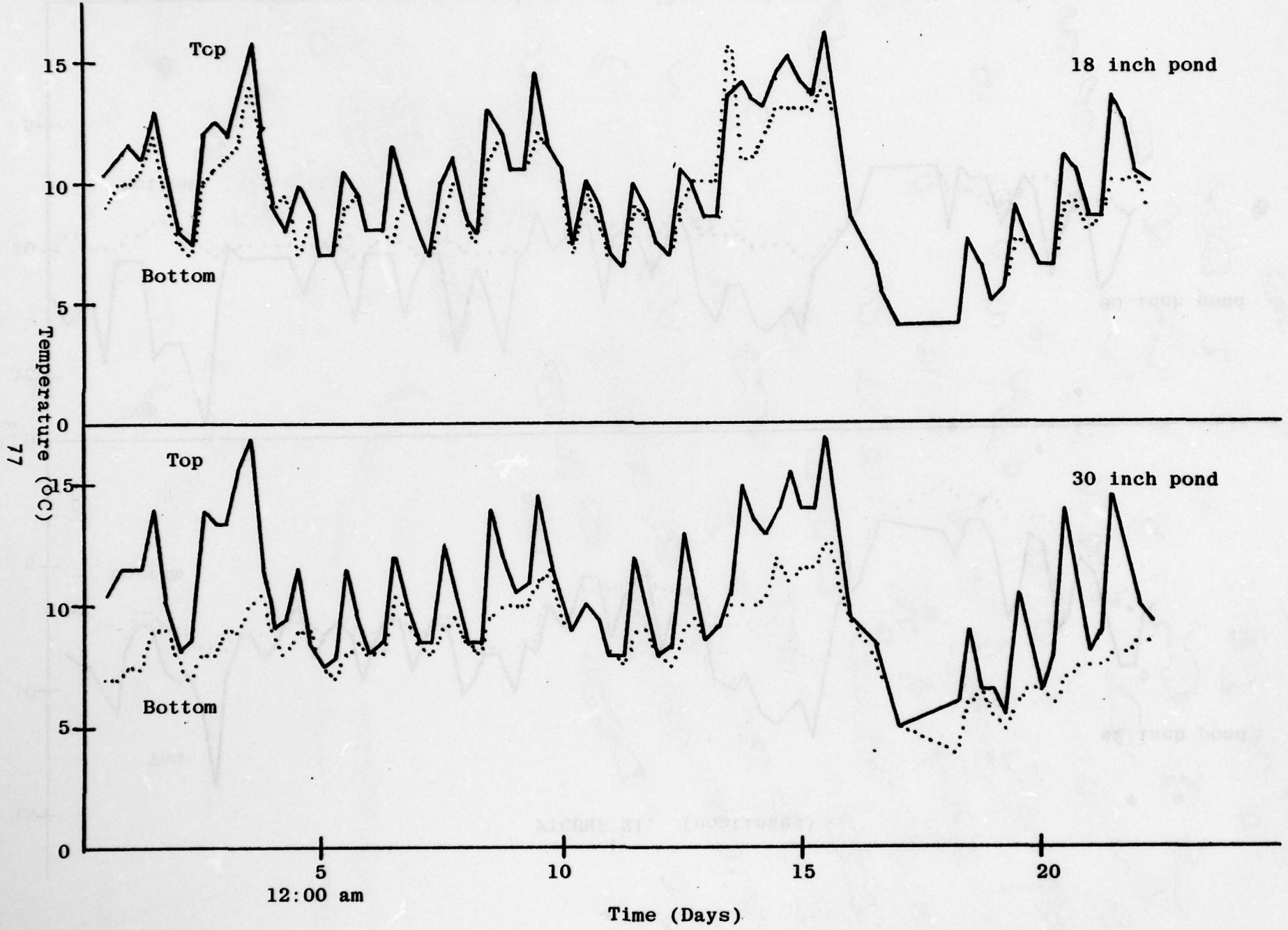
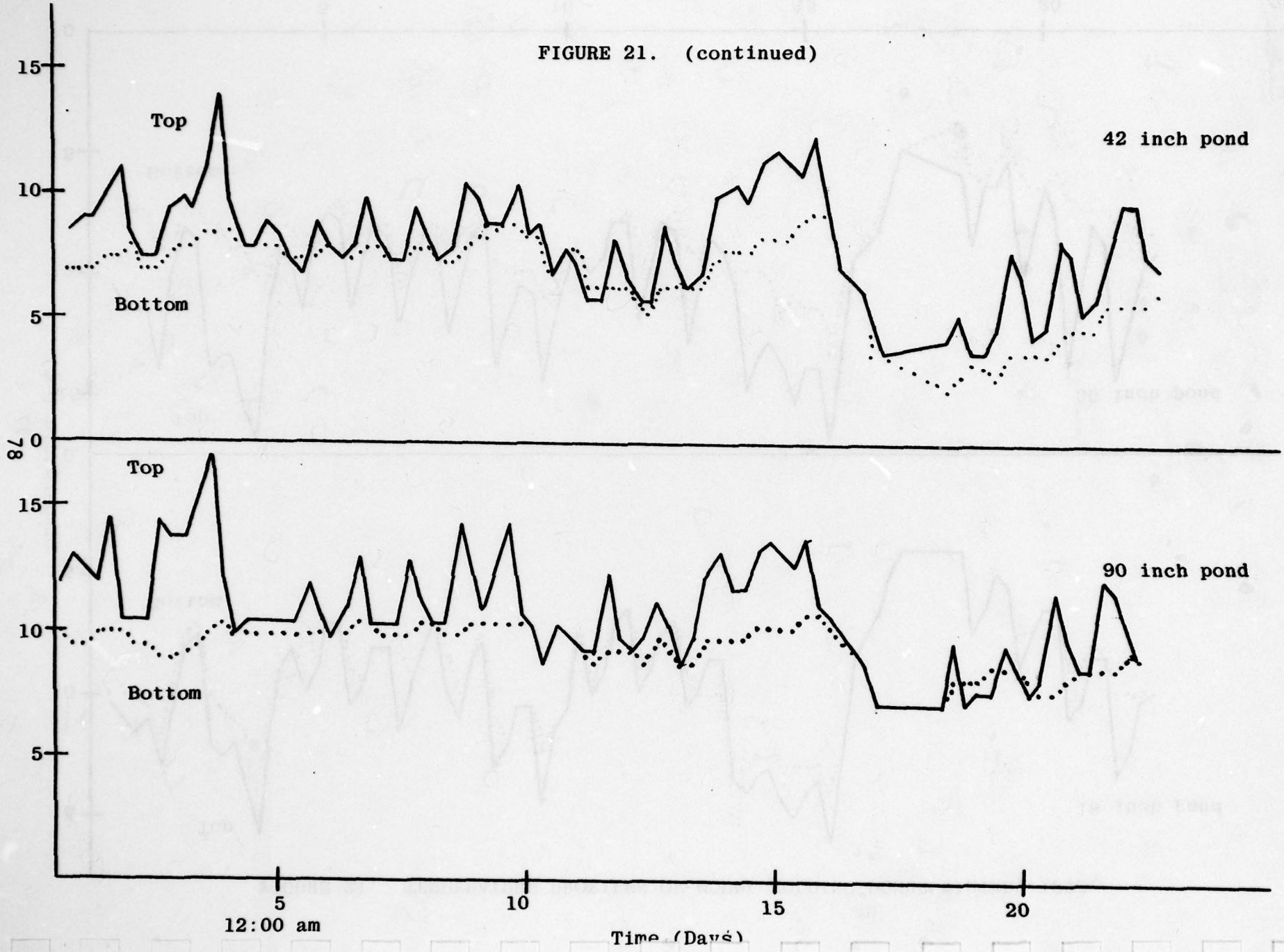


FIGURE 21. (continued)



respond to diurnal changes in ambient temperatures. It took an extended cold period to change temperatures appreciably. It can be seen that the mean temperatures for the 42-inch pond are lower than those for the 18-, 30-, and 90-inch ponds. This may be due to a systematic error in the temperature probes or recording device, as the probes were only calibrated before the field test began and not checked during the course of the experiment.

The survival of poliovirus as a percent of the total recovered at sample day one is illustrated in FIGURES 22 and 23. The ponds showed little or no real difference in virus survival between the bottom and top wastewater sample for the test period. The appearance of algae and the consequent elevation of pH (TABLES 17 and 18) seems to have accelerated the viral decay rate in the 42-inch and 90-inch ponds during the latter portion of the test. (Compare die-off between 0-17 days and 17 days through the end of the study period).

It is clear that the rate of virus inactivation during this test series is much lower than during the Fall, 1975 series. Temperature contributed to this difference as pond temperatures during the winter were around 10C, whereas those in the fall were close to 20C.

TABLE 19 is a summary of viral isolations in sediments of each of the test ponds at the end of this run. Although virus in the overlying waters was below the practical limits of detection after 30 days, there were 41 pfu isolated per gram of sediment from one of the 30-inch ponds at 67 days.

TABLE 19. POLIOVIRUS ISOLATED IN POND SEDIMENTS, WINTER, 1976.

Pond Depth (inches)	Sampling Time (days)	Virus isolated from Sediment (pfu/gm)
18	67	1
30	67	41
42	67	3
90	60	50

TABLE 17. pH OF POND SERIES 1, WINTER, 1976.

Time After Virus Addition (days)	18"	30"	42"	90"
0	7.6	7.7	7.7	7.7
1	7.8	7.7	7.7	7.6
3	7.5	7.6	7.5	7.6
6	7.4	7.4	7.5	7.4
10	7.6	7.6	7.4	7.1
13	7.6	8.1	7.5	7.3
17	8.3	8.9	7.6	7.6
24	8.9	8.9	8.	7.6
31	9.0	8.5	8.5	8.1
38			8.9	8.7
45			9.5	8.7

TABLE 18. pH OF POND SERIES 3, WINTER, 1976.

Time After Virus Addition (days)	18"	30"	42"	90"
0	7.6	7.8	7.8	7.6
1	7.7	7.8	7.8	8.0
3	7.5			
6	7.5	7.5	7.6	7.7
10	7.5	7.5	7.5	7.6
13	7.8	7.8	7.5	7.8
17	8.5	9.1	7.8	8.1
24	8.9	9.0	8.3	8.0
31	9.1	8.8	8.7	8.3
38			9.0	8.6
45			9.1	8.8

FIGURE 22. POLIOVIRUS SURVIVAL IN SERIES 1 FINAL EFFLUENT PONDS-WINTER, 1976.

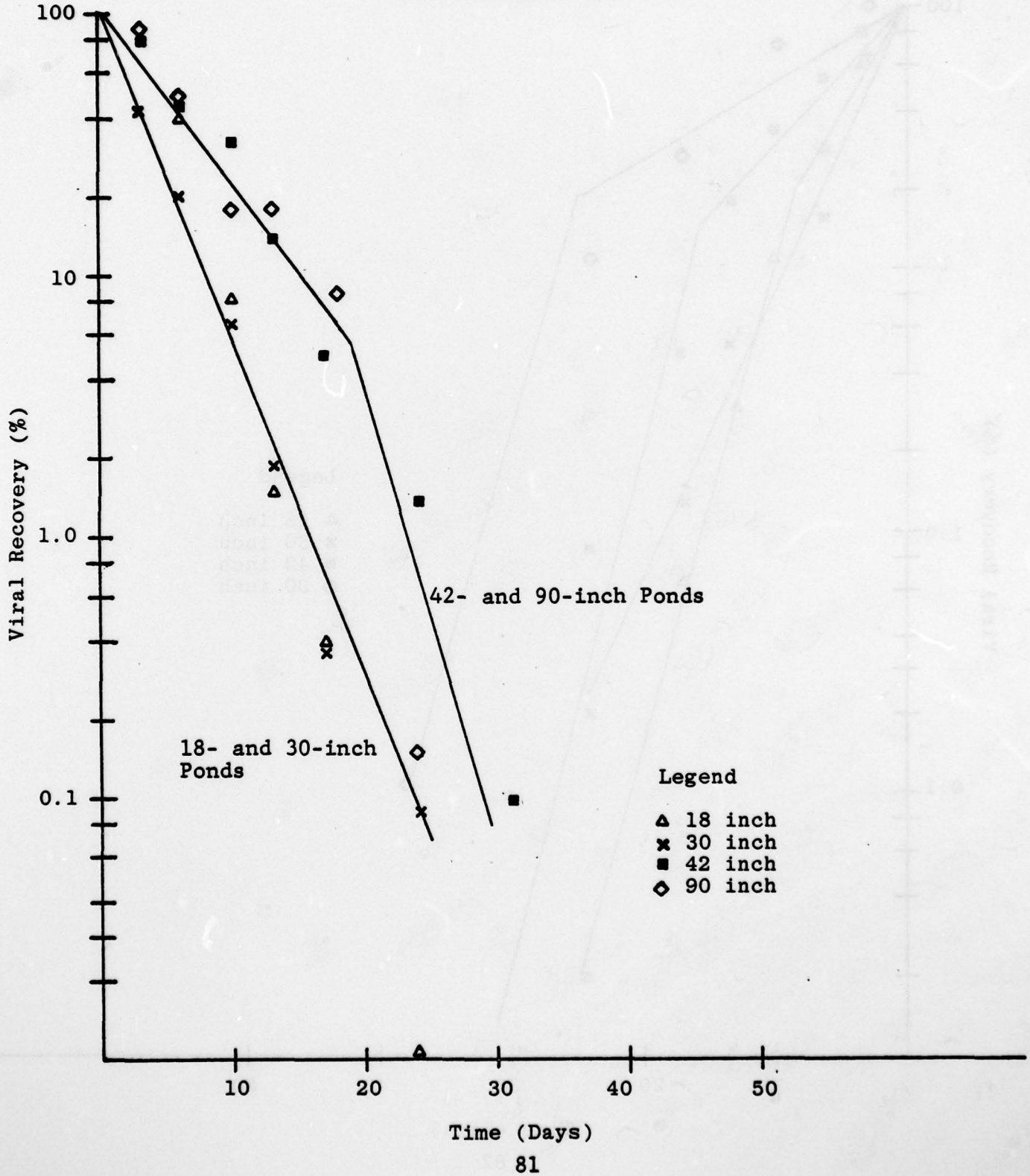
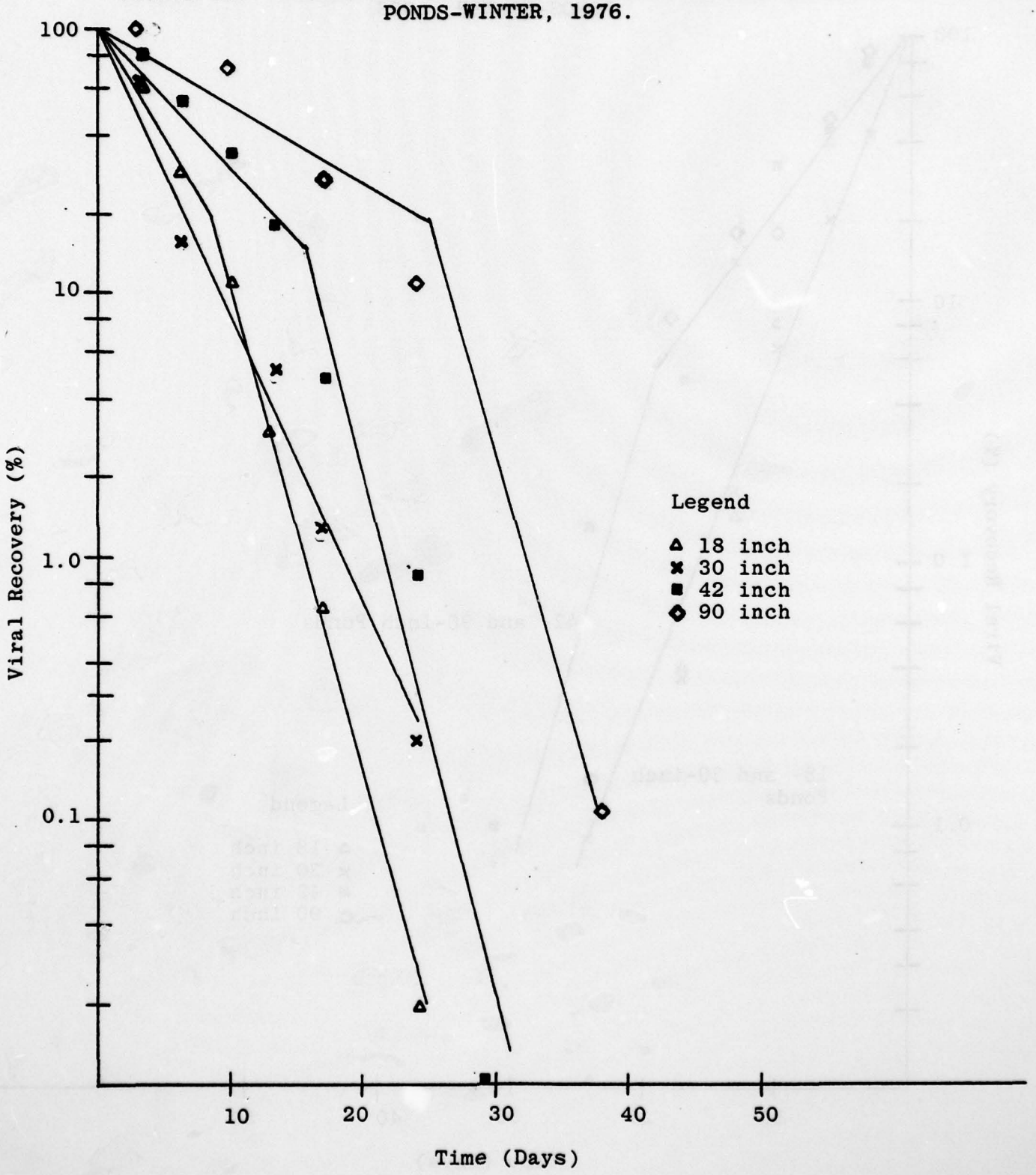


FIGURE 23. POLIOVIRUS SURVIVAL IN SERIES 3 FINAL EFFLUENT PONDS-WINTER, 1976.



Spring Test Series, 1976

This field test was begun in March using one pond of each depth filled with final effluent and one 30-inch pond and one 90-inch pond filled with primary effluent. Viral activity and chemical and physical parameters were monitored as described previously. Additionally, the accumulation of sediment and the presence of virions therein was observed throughout this test series.

The percentage of recoverable virions based on the one hour sampling time is presented in FIGURES 24 and 25. It can be seen that loss from the liquid phase of the final effluent ponds is relatively rapid; all have lost at least 99.9% of the added infectious virions within 20 days. Loss of recoverable virus was much slower in the primary effluent ponds where 10% and 4% of the virions were present after 35 days in the 90-inch and 30-inch ponds respectively.

The rapid loss of virus from the final effluent ponds was due, in part, to temperature (TABLE 20). However, an examination of FIGURE 24 indicates that the rate of inactivation increased, at least in the 42-and 90-inch ponds between 6 and 15 days. Concurrent with the increased inactivation rate of poliovirus in these two ponds was an elevation in pH (TABLE 21). In the 18-and 30-inch ponds an algal community was established quickly and pH became high almost from the onset of the test. In contrast to the final effluent, the pH of the primary effluent ponds remained fairly low over the entire test period.

TABLE 20. TEMPERATURE AVERAGES (%) IN PONDS, SPRING, 1976.

Time (days)	18"	30"	42"	90"
0	16	18	19	20
1	14	16	16	18
15	21	22	20	19
17	21	20	18	18
19	17	18	17	17
21	19	20	18	18
23	20	20	20	19
25	22	22	21	21
27	22	22	19	19

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THE SURVIVAL OF HUMAN ENTERIC VIRUSES IN HOLDING PONDS.(U)
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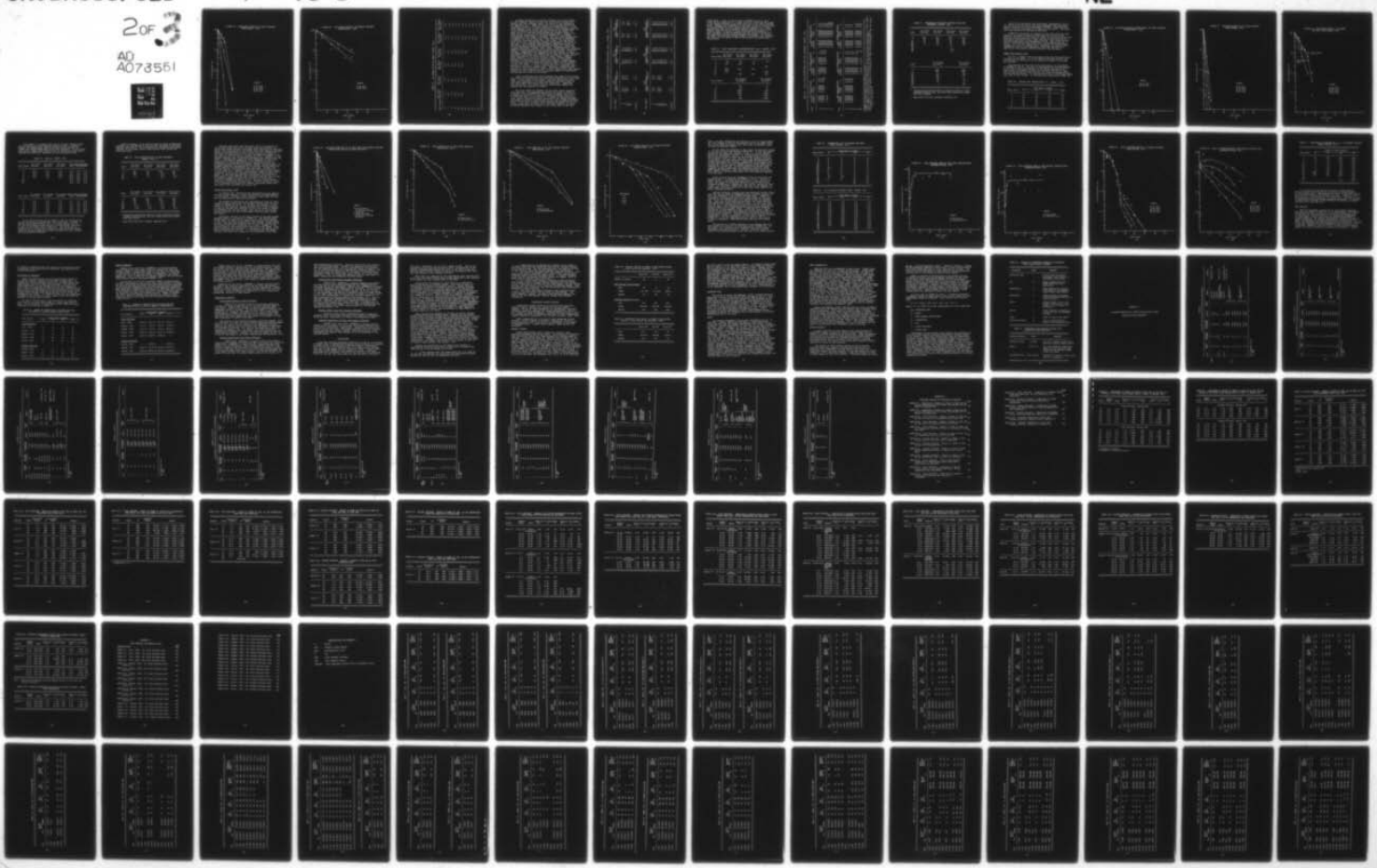


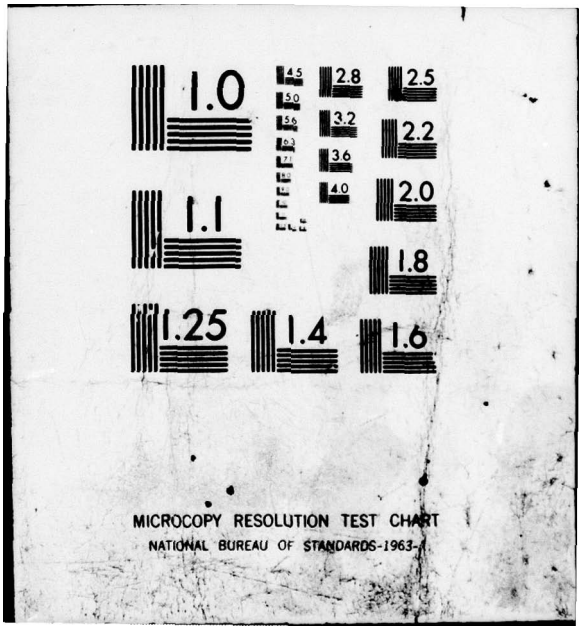

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

FIGURE 24. POLIOVIRUS SURVIVAL IN FINAL EFFLUENT PONDS-SPRING, 1976.

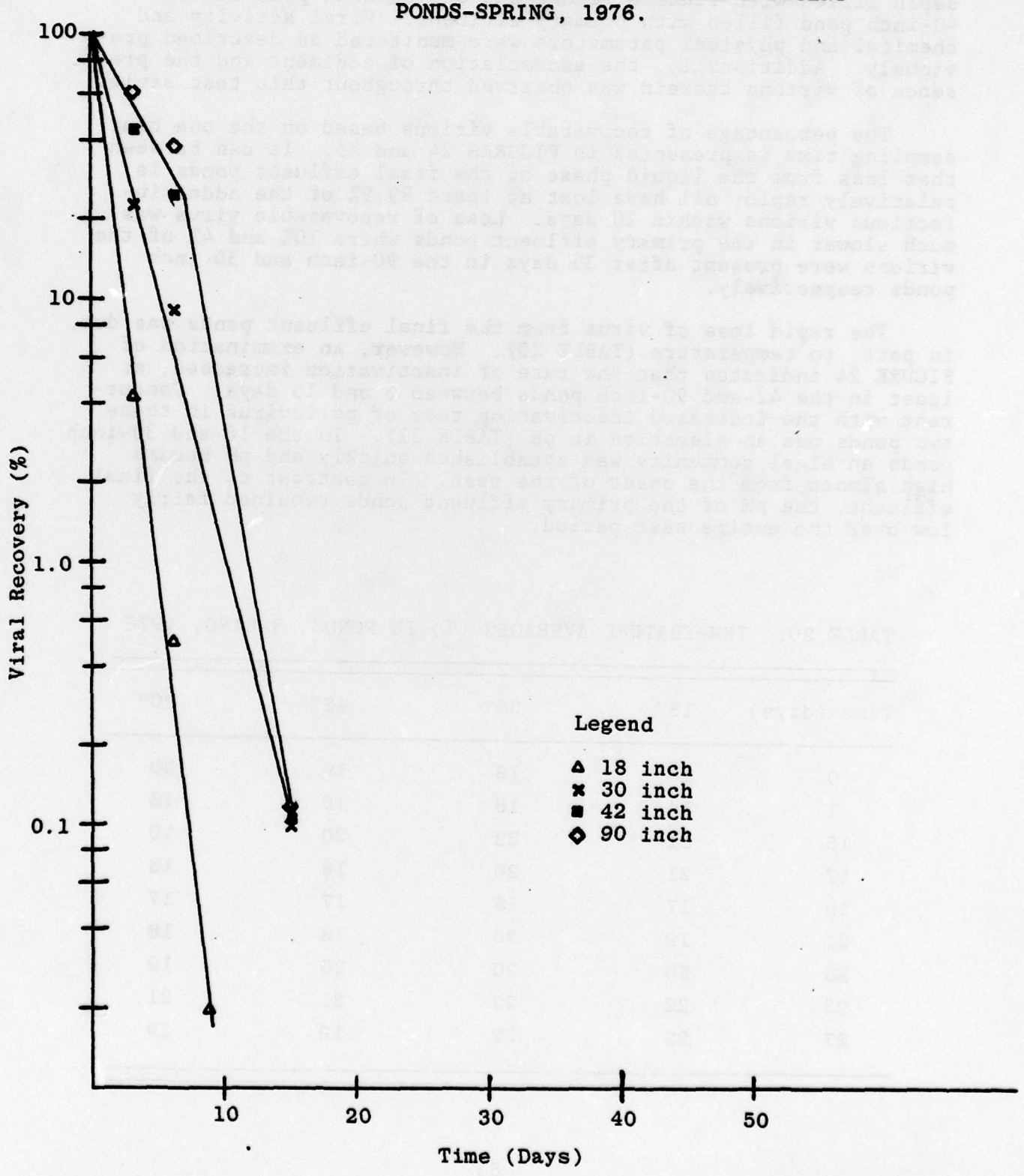


FIGURE 25. POLIOVIRUS SURVIVAL IN PRIMARY EFFLUENT PONDS-SPRING, 1976.

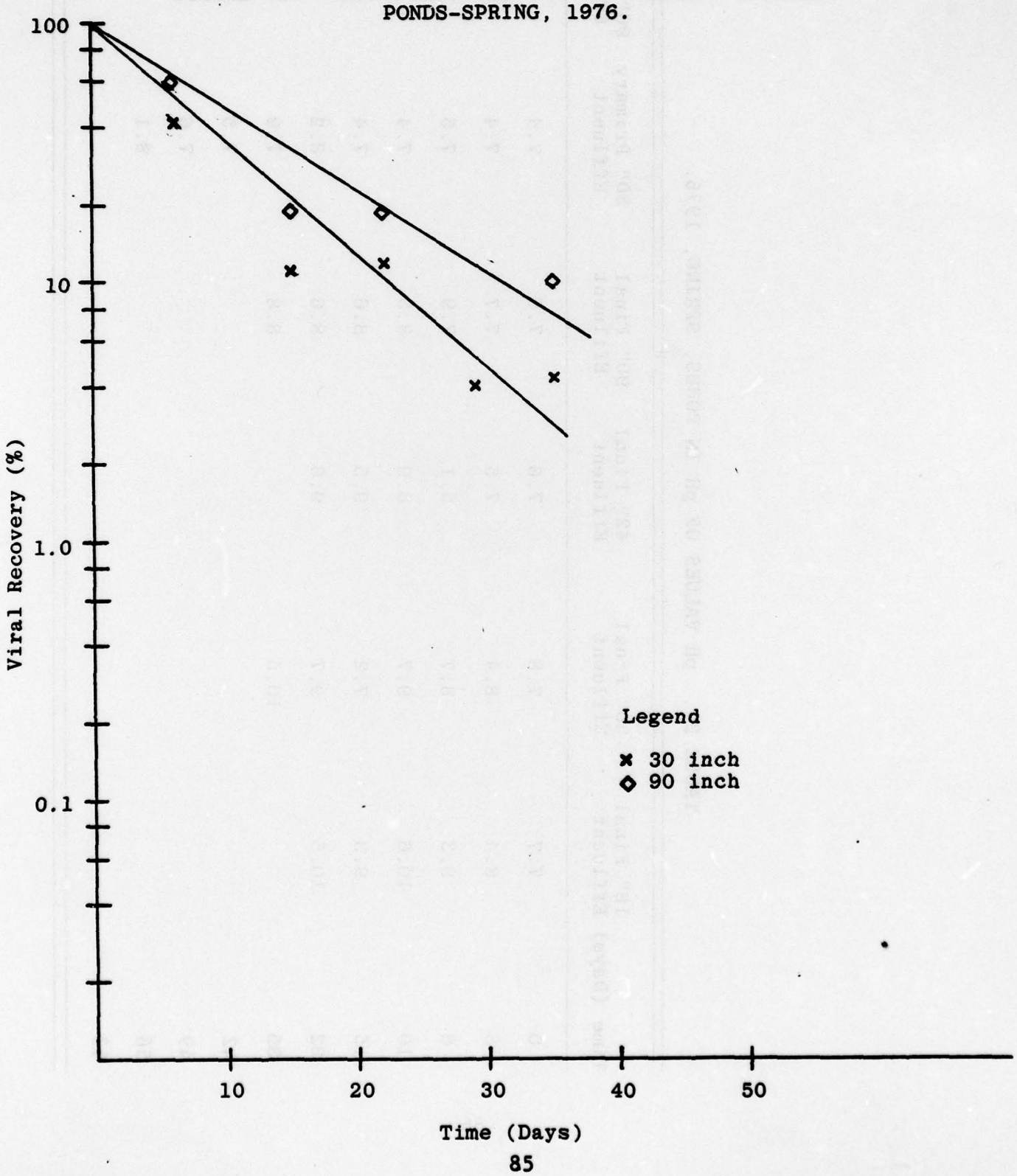


TABLE 21. pH VALUES OF pH IN PONDS, SPRING, 1976.

Time (Days)	18" Final Effluent	30" Final Effluent	42" Final Effluent	90" Final Effluent	30" Primary Effluent	90" Primary Effluent
0	7.7	7.8	7.6	7.5	7.4	7.2
3	8.4	8.4	7.5	7.7	7.4	7.3
6	9.3	8.7	8.1	7.9	7.5	7.3
10	10.6	9.7	8.9	8.5	7.4	7.3
15	9.3	7.2	9.5	8.6	7.4	7.2
22	10.5	9.7	9.6	8.6	8.2	7.6
35		10.5		8.8	7.9	7.7
42					7.5	7.7
49					7.6	7.8
56					8.1	7.5
63						7.8

Although differences in pH were probably one of the reasons for the different rates of poliovirus inactivation between the primary and final effluent ponds, the nature of the wastewaters was important also. TABLE 22 presents the suspended solids concentrations found in the ponds during the testing period. In the final effluent ponds, solids concentrations were initially high due to the suspension of solids from the bottom of the backfilled ponds into the overlying liquid during the mixing process. As these settle out the solids concentrations go down. However, as algae became established in the ponds (TABLE 23), the amount of suspended solids began to increase, and this trend predominated throughout the rest of the test. This observation is reflected in volatile suspended solids (VSS) data, also. For the shallow ponds there was a significantly higher percentage of VSS later in the runs. In the primary effluent ponds, again high solids concentrations were observed after mixing followed by a rapid drop in this measurement between 0 and 3 days. Suspended solids then remained relatively constant until around 22 days when the increasing numbers of algae began to raise solids concentrations. Therefore, although suspended solids concentrations were similar in final and primary effluents, the composition of these solids was different, especially during the first 2/3 of the test period. Algae are a major component of the final effluent solids, whereas sewage particulates are the primary source of solids in the primary effluent. As observed in the laboratory studies, viruses appear to associate with the sewage particulates found in final effluent but not with algae. If this is indeed the case, the protection afforded to viruses by sewage solids would not be available where the solids levels are largely algal. Therefore, another factor involved in the differential rates of virus inactivation seen between primary and final effluent may be a greater degree of association with protective solids in the former.

Virus association with solids implies that some of the virions will settle to the bottom of the pond and be deposited in the sludge layer. It was shown in the Winter, 1976 Test Series that poliovirus was recoverable in the pond sediments long after those in the liquid phase were below detection sensitivity. Beginning with the Spring, 1976 series, settled solids were sampled and assayed for the presence of viruses.

The total amount of sediment and the virus levels recovered from them over a 10-week sampling period are listed in TABLE 24. It appears that sedimentation takes place throughout this period, although the majority of solids are deposited within the first 1 to 2 weeks after virus addition. It cannot be determined from these data if viruses also were deposited in the sludge throughout the sampling period as inactivation occurs even as deposition takes place. It is clear, however, that although the number of viruses in the sludge does not exceed 6% of the total added to any pond, poliovirus persists much longer in the solids than in the over-

TABLE 22. POND SUSPENDED SOLIDS CONCENTRATIONS (mg/l), SPRING, 1976.

Time (days)	18" Final Effluent		30" Final Effluent		42" Final Effluent	
	Total	Volatile	Total	Volatile	Total	Volatile
0	73.3	27.9	130.4	36.1	20.2	11.4
3	91.1	11.5	36.1	17.5	9.1	4.5
6	54.9	41.7	39.2	28.0		
15	62.8	51.0	70.9	50.6	76.2	38.6
22			77.5	62.3	64.1	50.0
35			79.0	60.0		
Average	70.5	33.0	72.1	42.4	42.4	26.1

Time (days)	90" Final Effluent		30" Primary Effluent		90" Primary Effluent	
	Total	Volatile	Total	Volatile	Total	Volatile
0	19.4	10.3	132.2	58.7	148.1	98.3
3	9.5	6.4	54.1	38.6	46.0	37.9
6			41.3	27.0	32.9	23.3
15	27.3	21.8	37.4	30.3	31.9	28.3
22	52.8	49.6	53.2	42.5	62.8	50.7
29	54.4	34.2	28.0	21.8	47.4	31.5
34			67.7	62.7	94.1	85.9
43			26.3	22.9	53.3	43.1
50			62.5	36.2	54.0	38.9
Average	32.7	40.8	59.1	37.8	63.4	48.6

lying liquid. Also, as can be seen in TABLES 24 and 25, more virus can be recovered for a longer period of time from the sludge of the primary effluent ponds than from that of the final effluent ponds. Whether this difference in recovery is due to a faster rate of virus movement into the sludge from the liquid or to a lower inactivation rate in the primary effluent sediments in not clear. However, from the nature of virus inactivation in primary vs. final effluent, it can be supposed that the solids from the primary wastewater provide more protection for viruses. If this is the case, then the pond sludges formed by different type effluents may differ in the degree to which they protect poliovirus from inactivation.

TABLE 23. POND CHLOROPHYLL CONCENTRATIONS (µg/1), SPRING, 1976.

Time (days)	18" Final Effluent	30" Final Effluent	42" Final Effluent	90" Final Effluent
0	40	50	10	10
3	270	270	20	
6				240
8	1230	1110	722	
14	250			900
22		750	590	850

Time (days)	30" Primary Effluent	90" Primary Effluent
0	110	10
6	20	10
14	4480	100
22	1850	1220
28	80	1370
35	730	6780

TABLE 24. TOTAL VIRUS RECOVERABLE FROM POND SEDIMENTS, SPRING, 1976.

Time[α]	18" Final Effluent			30" Final Effluent			42" Final Effluent		
	Total Sediment[ρ]	Total Virus[υ]	%[σ]	Total Sediment	Total Virus	%	Total Sediment	Total Virus	%
1	$1.0X10^5$	$2.4X10^7$	0.06	$9.9X10^4$	$7.0X10^8$	1.1	$8.4X10^4$	$5.5X10^8$	0.73
2	$1.5X10^5$	$4.4X10^6$	0.01	$1.8X10^5$	$6.9X10^8$	1.0	$8.6X10^4$	$2.8X10^8$	0.37
3	$2.6X10^5$	$9.5X10^6$	0.02	$1.5X10^5$	$6.1X10^8$	0.92	$1.5X10^8$	$1.3X10^8$	0.17
4	$1.4X10^5$	$1.1X10^6$	0.003	$7.6X10^4$	$9.1X10^7$	0.14	$1.3X10^5$	$2.5X10^7$	0.03
5				$2.2X10^5$	$1.8X10^8$	0.27	$1.4X10^5$	$5.5X10^7$	0.07
6	$2.5X10^5$	--	--				$2.4X10^5$	$3.1X10^7$	0.04
7							$2.5X10^5$	$2.5X10^5$	0.003
8							$2.9X10^5$	$1.6X10^6$	0.002
9							$4.8X10^5$	$1.4X10^6$	0.002

Time	18" Final Effluent			30" Primary Effluent			90" Primary Effluent		
	Total Sediment	Total Virus	%	Total Sediment	Total Virus	%	Total Sediment	Total Virus	%
1	$8.8X10^4$	$1.3X10^9$	0.76	$8.5X10^4$	$1.6X10^9$	2.3	$1.2X10^5$	$3.6X10^9$	1.9
2	$9.0X10^4$	$9.9X10^8$	0.58	$1.0X10^5$	$4.4X10^9$	6.4	$2.2X10^5$	$8.6X10^9$	4.5
3	$1.3X10^5$	$3.9X10^8$	0.23	$1.6X10^5$	$1.3X10^9$	1.9	$2.4X10^5$	$2.4X10^9$	1.3
4	$2.2X10^5$	$2.9X10^8$	0.18	$1.3X10^5$	$7.0X10^8$	1.0	$3.4X10^5$	$3.3X10^9$	1.7
5	$2.5X10^5$	$3.0X10^8$	0.18	$3.1X10^5$	$2.2X10^9$	3.2	$4.4X10^5$	$6.2X10^9$	3.3
6				$3.9X10^5$	$1.2X10^9$	1.7			
7				$3.2X10^5$	$9.3X10^8$	1.3	$4.1X10^5$	$1.5X10^9$	0.79
8				$5.5X10^5$	$4.2X10^8$	0.61	$5.1X10^5$	$1.3X10^8$	0.07
9							$6.0X10^5$	$1.1X10^9$	0.58
10				$7.0X10^5$	$6.0X10^7$	.09	$6.1X10^5$	$6.7X10^8$	0.35

α Number of weeks after virus addition to pond.
ρ Total amount of sediment (mg) accumulated at bottom of pond extrapolated from a single sample.
υ Total number of poliovirus (pfu) in sediment extrapolated from a single sample.
σ The number of pfu in the sediment as a percentage of those virus recoverable from the liquid 1 hour after addition.

TABLE 25. PERCENTAGE POLIOVIRUS RECOVERY FROM POND SEDIMENTS, SPRING, 1976.

Time[α]	18" Final Effluent	30" Final Effluent	42" Final Effluent	90" Final Effluent
0	100	100	100	100
7	18	99	51	76
14	40	87	24	30
21	5	13	5	22
28	0	26	10	23
35			6	
42			1	
49			0.3	
56			0.3	

Time[α]	30" Primary Effluent	90" Primary Effluent
0	100	100
7	275	238
14	81	67
21	44	92
28	138	172
35	75	
42	58	42
49	26	4
56		31
63	4	19

* Percentage calculated based on pfu/gm recovered at first sediment sampling time, one hour after ponds were seeded with test viruses.

α Days after the first sediment sampling time.

Thirty-five days after test initiation, approximately 33% of the liquid in the 30-and 90-inch final effluent ponds was pumped out and replaced with fresh wastewater effluent. Poliovirus was added to a final concentration of about 1 X 10^4 pfu/ml. The ponds were then monitored as previously described.

FIGURE 26 illustrates the removal of the virus from the pond waters as a percent of those virions detected one hour after addition. The inactivation rates are rapid; no virus was recoverable from the 30-inch pond after 8 days, and the 90-inch pond had no detectable poliovirus 15 days after addition. Both ponds already had well-established algae communities at the time of partial refill. Over the test period the pH in the 30-and 90-inch ponds averaged 9.3 and 8.3, respectively. Therefore, an alkaline pH in combination with daytime temperatures near 20C resulted in a rapid loss of poliovirus from the liquid of the two ponds.

Summer Test Series, 1976

In July, a summer field test began using both test wastewaters in ponds at each depth. As in the Spring, 1976 Test Series, the inactivation of poliovirus in the liquid and sludge of the ponds was followed.

The survival of the virus in the pond waters is illustrated in FIGURES 27 and 28. The rate of inactivation in both wastewaters is more rapid than that observed in the Spring Test Series (i.e., there were no recoverable virions in the final effluent in less than 10 days and 99% of the virus was lost from the primary effluent ponds within 20 days). The rapid inactivation of poliovirus during this test is due, in part, to the high pond temperatures (TABLE 26).

TABLE 26. AVERAGE POND TEMPERATURES (^{o}C), Summer, 1976.

	Pond Depth (inches)			
Time (days)	18	30	42	90
1	30	29	28	26
3	30	29	27	26
4	28	28	26	25
5	28	28	26	25
6	29	28	26	25

FIGURE 26. POLIOVIRUS SURVIVAL AFTER REFILL OF FINAL EFFLUENT PONDS-SPRING, 1976.

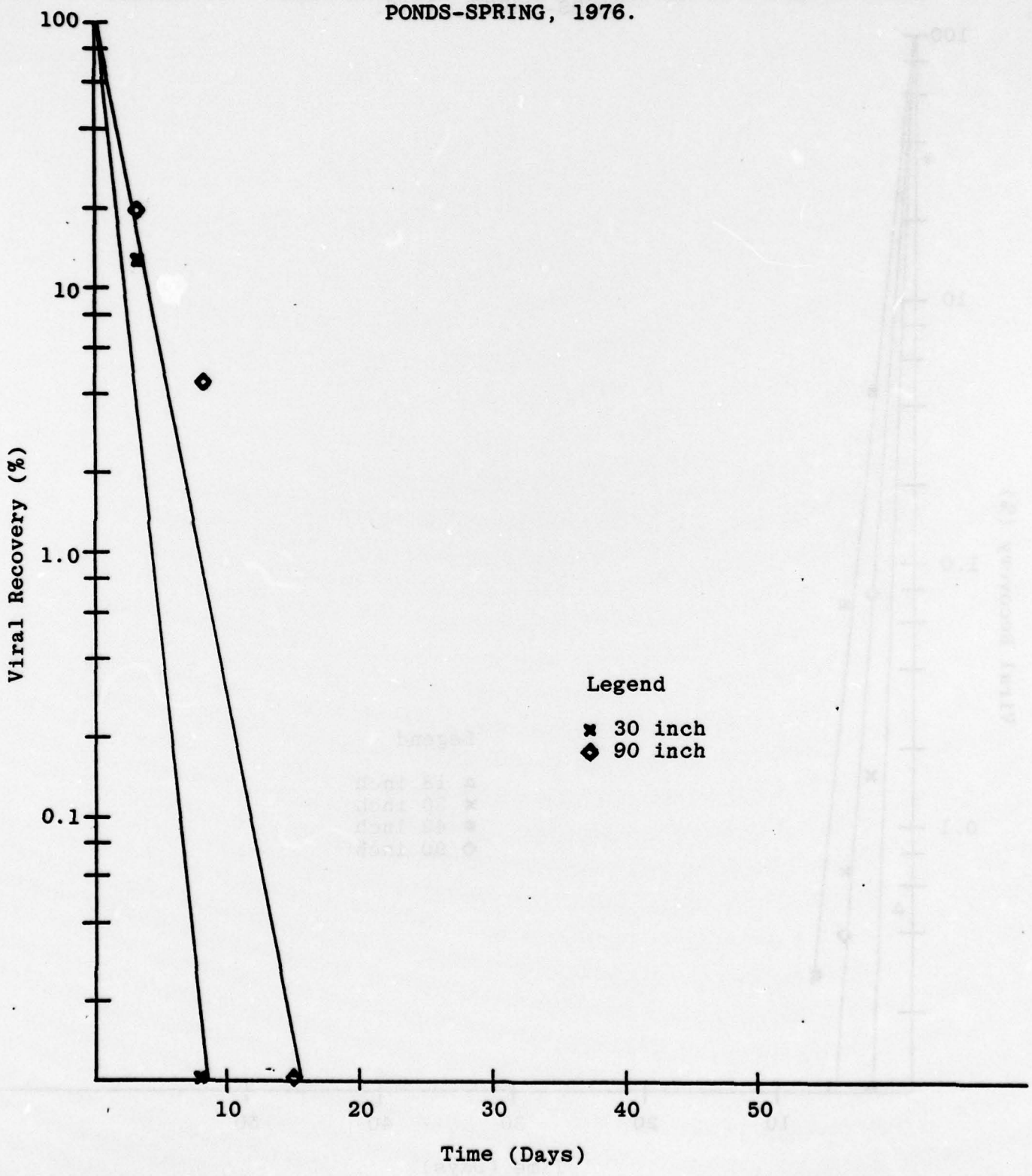


FIGURE 27. POLIOVIRUS SURVIVAL IN FINAL EFFLUENT PONDS-SUMMER, 1976.

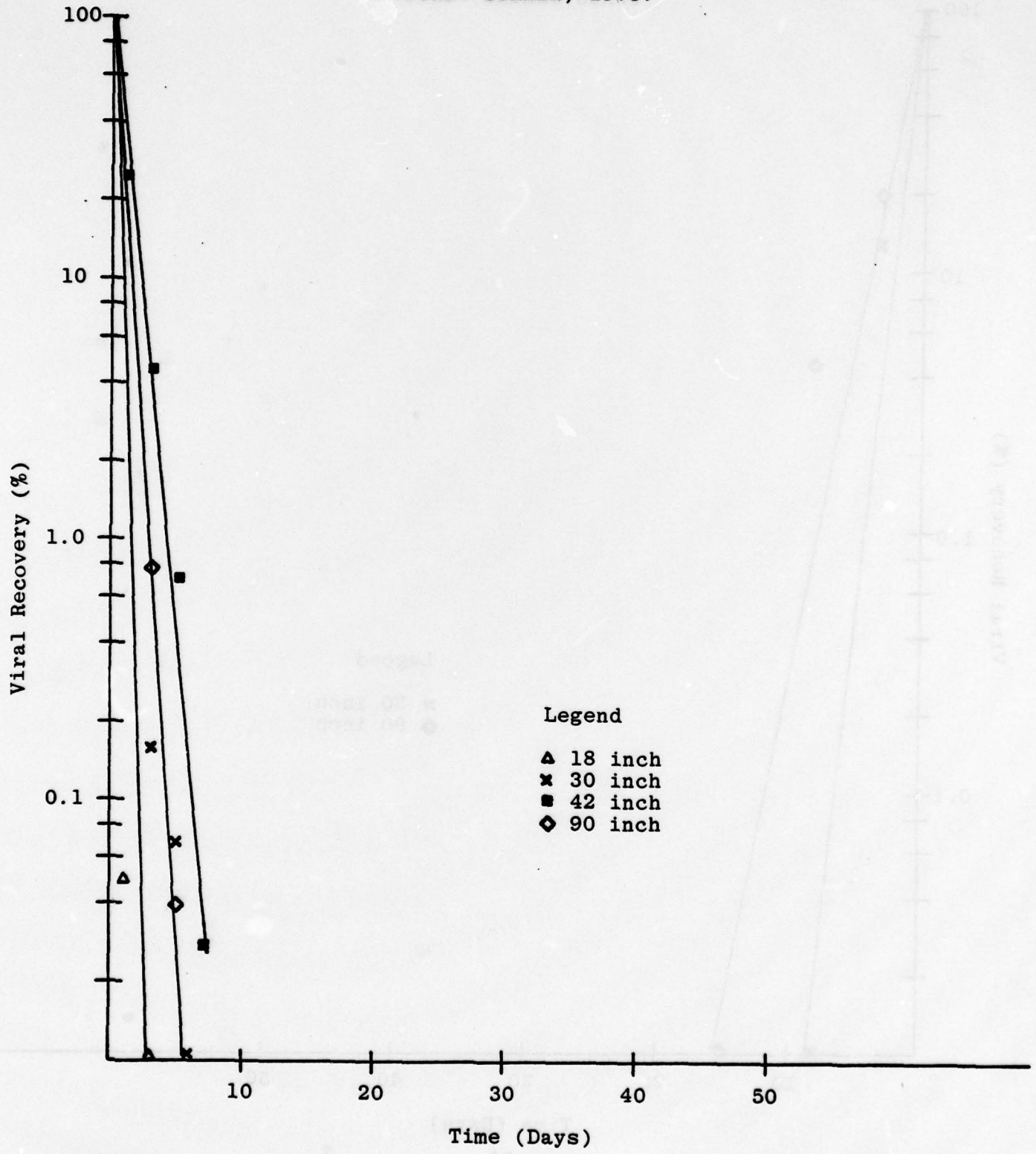
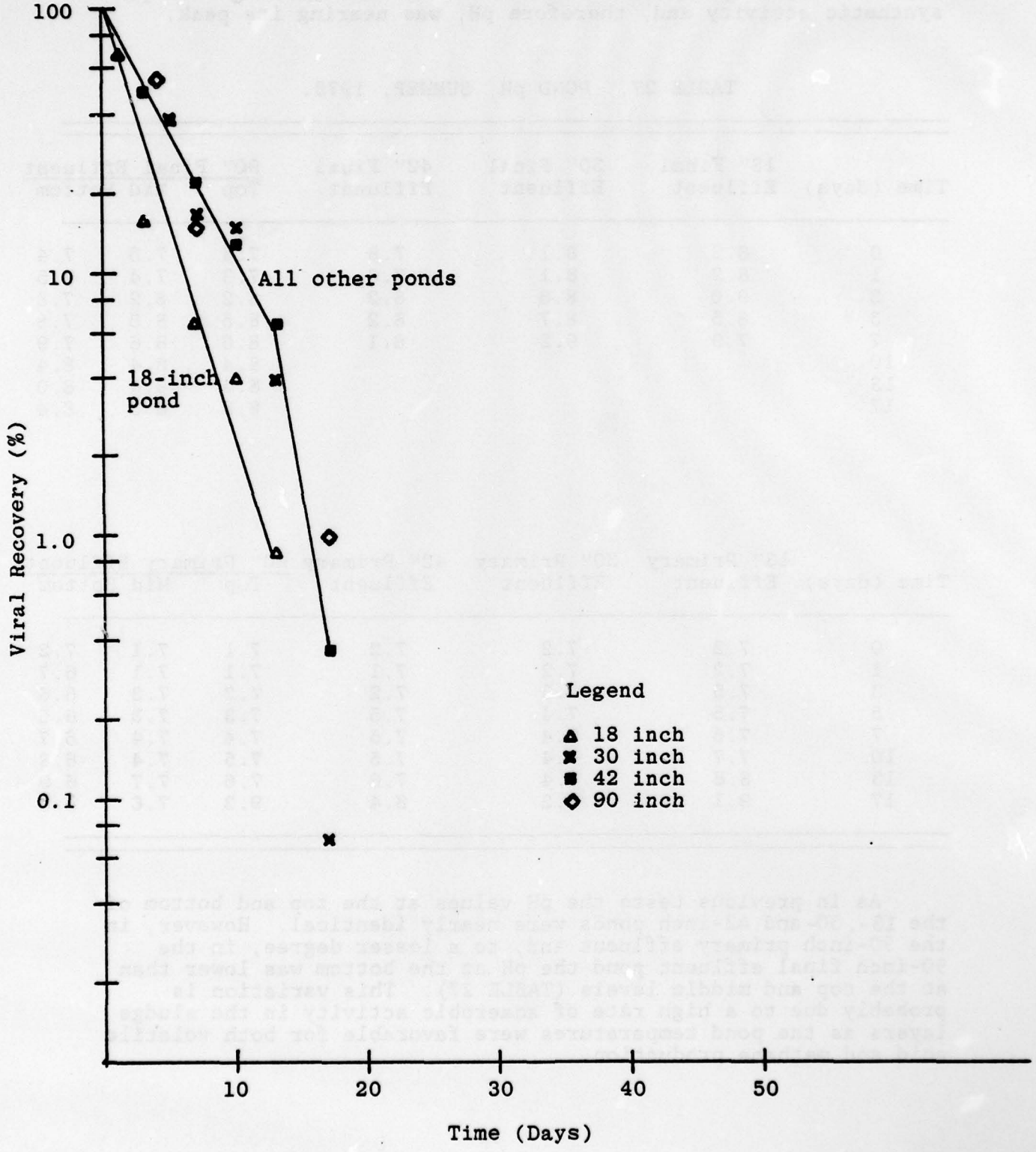


FIGURE 28. POLIOVIRUS SURVIVAL IN PRIMARY EFFLUENT PONDS-SUMMER, 1976.



From TABLE 27 it appears that the pH of final effluent ponds did not reach the levels attained during the spring, probably because the samples were taken soon after sunrise during the summer and photosynthesis was not yet well underway. In the Spring Test Series, samples were taken at mid to late morning when photosynthetic activity and, therefore pH, was nearing its peak.

TABLE 27. POND pH, SUMMER, 1976.

Time (days)	18" Final Effluent	30" Final Effluent	42" Final Effluent	90" Final Effluent		
				Top	Mid	Bottom
0	8.2	8.1	7.8	7.4	7.5	7.4
1	8.2	8.1	7.6	7.3	7.4	7.5
3	9.0	8.6	8.2	8.2	8.2	7.8
5	8.5	8.7	8.2	8.5	8.5	7.8
7	7.9	9.2	8.1	8.6	8.6	7.9
10				8.4	8.4	8.4
13				8.4	8.4	8.0
17				8.8	8.3	8.4

Time (days)	18" Primary Effluent	30" Primary Effluent	42" Primary Effluent	90" Primary Effluent		
				Top	Mid	Bottom
0	7.2	7.2	7.2	7.1	7.1	7.2
1	7.2	7.2	7.1	7.1	7.1	6.7
3	7.5	7.3	7.2	7.2	7.3	6.6
5	7.5	7.4	7.5	7.3	7.3	6.6
7	7.5	7.4	7.5	7.4	7.4	6.7
10	7.7	7.4	7.5	7.5	7.4	6.8
13	8.6	7.4	7.6	7.6	7.7	6.9
17	9.1	8.2	8.4	9.3	7.6	6.6

As in previous tests the pH values at the top and bottom of the 18-, 30- and 42-inch ponds were nearly identical. However, in the 90-inch primary effluent and, to a lesser degree, in the 90-inch final effluent pond the pH at the bottom was lower than at the top and middle levels (TABLE 27). This variation is probably due to a high rate of anaerobic activity in the sludge layers as the pond temperatures were favorable for both volatile acid and methane production.

TABLE 28 presents virus recovery from the model holding pond sediments as a percentage of those virions recovered at the first sampling time (Day 3). As was observed in the Spring Test Series, survival in the sludge layer is much greater than in the overlying pond water.

TABLE 28. POLIOVIRUS SURVIVAL IN POND SEDIMENTS, SUMMER, 1976. *

Time[α]	18" Final Effluent	30" Final Effluent	42" Final Effluent	90" Final Effluent
0	100	100	100	100
7	147	14	2.8	65
10	75	51	3.4	12
13	0	0	3.0	9.0
21			0	

Time[α]	18" Primary Effluent	30" Primary Effluent	42" Primary Effluent	90" Primary Effluent
0	100	100	100	100
7	120	100	113	69
10	75	23	9.0	20
13	33	21	30	12
21	9.0	3.4	4.5	20
28	1.1	1.8	3.1	14
35	0.75	1.3	1.8	2.2

* Percentage calculation based on pfu/gm recovered at first sediment sampling time, one hour after ponds were seeded with test virus.

α Days after the first sediment sampling time.

Nineteen days after test initiation 33% of the contents of the 90-inch primary and final effluent ponds were removed, replaced with fresh effluent, and seeded with poliovirus as described above. The inactivation of poliovirus after this refill is plotted alongside the inactivation after initial filling in FIGURE 29. The rates of inactivation in the final effluent pond appear to be the same for both the initial filling and refill. However, in the primary effluent pond, inactivation proceeds more rapidly after the refill. Although the reasons for this difference in virus survival are not clearly delineated, it is probably not due to temperature as the weather was similar during the refill test. By replacing only 33% of the original effluent, the biological and chemical conditions established in the initial filling would not become completely reestablished upon refilling. Evidence for this was seen in the generally lower COD, TOC and nitrogen levels in the refill test. Further, the microbial community was probably different, after refill, especially because of the presence of algae. It has been observed that inactivation of poliovirus is more rapid in final effluent than in primary effluent due, in part, to different degrees of antiviral activity in the microbial population (Sobsey and Cooper, 1973). The more rapid inactivation rate of poliovirus observed after refill is probably related to the different biological and chemical conditions within the pond after virus was added the second time.

Winter Test Series, 1977

In January 1977, poliovirus and Coxsackievirus were added to one pond at each depth at a final concentration of approximately 1×10^4 pfu/ml. The fate of both viruses in pond water and sediment was monitored as described previously.

The removal of poliovirus and Coxsackievirus from the water columns of the 42-inch final and primary effluent ponds is illustrated in FIGURES 30 and 31. It is clear that removal of both viruses is less rapid in primary effluent than in final effluent. It also can be seen that although initially the rates of removal are different for the two viruses, near the end of the testing period the percent of each virus removed from the water column was similar.

Poliovirus survival in the water columns of the four primary effluent ponds is illustrated in FIGURE 32. The shallow ponds showed about the same rate of inactivation in the first portion of the curve, all three losing 80% of the original viruses within 25 days. The deep pond departed from this scheme, as over 40% of the virions were still recoverable at 27 days. Changes in the initial rates of inactivation were seen in all four ponds. Once the inactivation rate increased, it appeared to be similar in all ponds, including the deep one. It is striking that the deeper the pond, the later the increase in the inactivation rate took

FIGURE 29. POLIOVIRUS SURVIVAL IN 90 INCH FINAL AND PRIMARY EFFLUENT PONDS AFTER INITIAL FILLING AND REFILL.

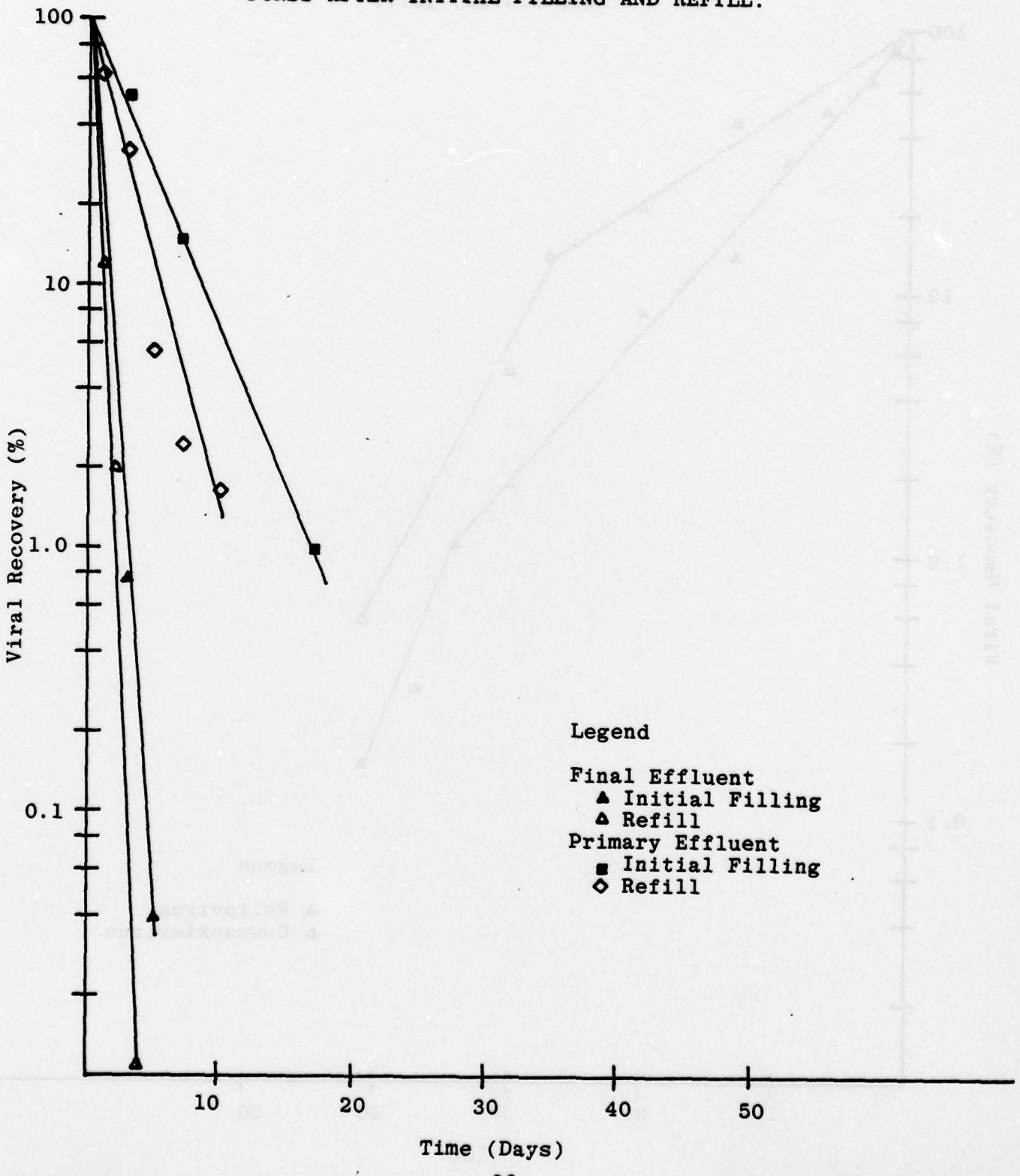


FIGURE 30. VIRUS SURVIVAL IN 42 INCH FINAL EFFLUENT POND-WINTER, 1977.

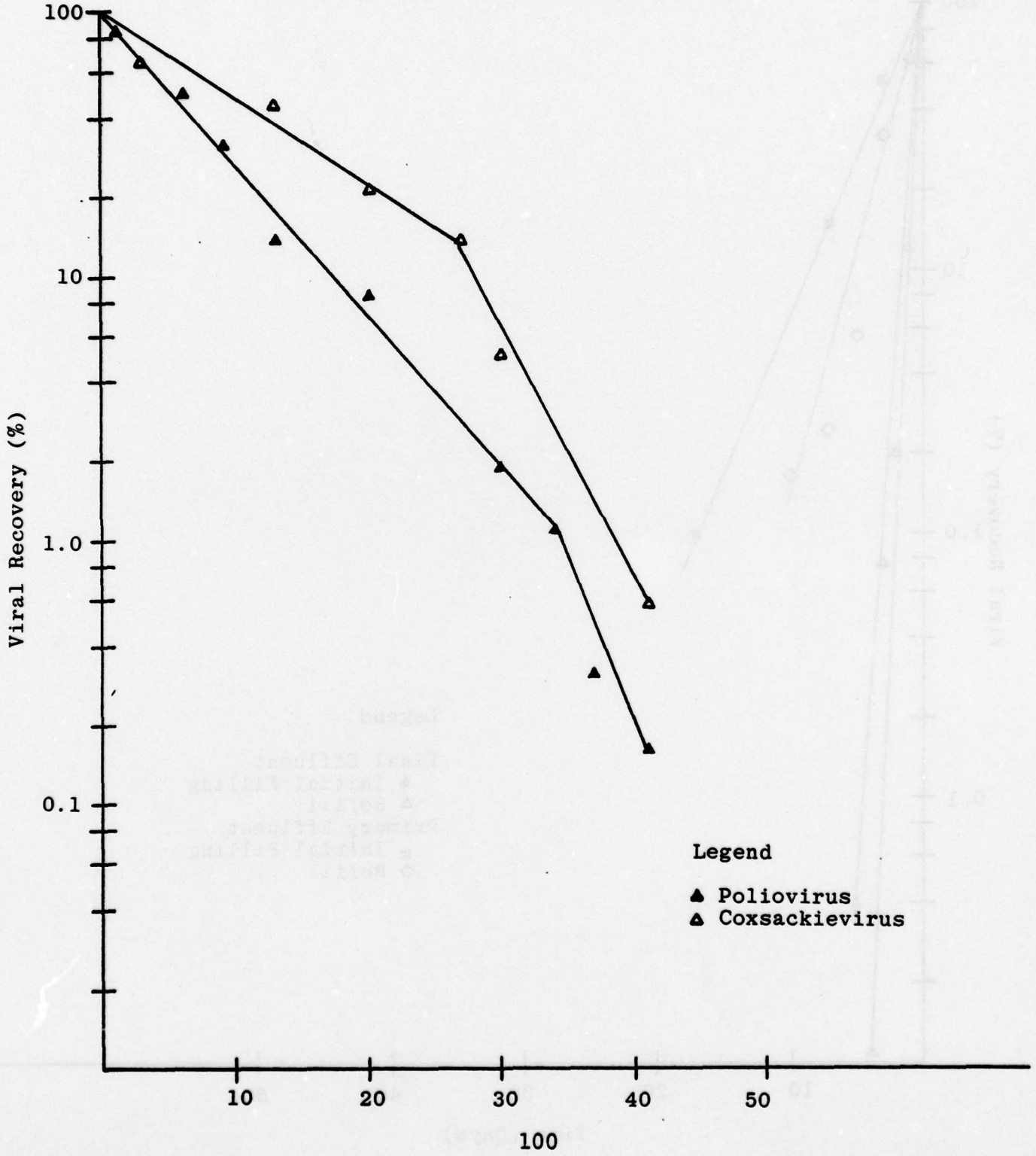


FIGURE 31. VIRUS SURVIVAL IN 42 INCH PRIMARY EFFLUENT POND-WINTER, 1977.

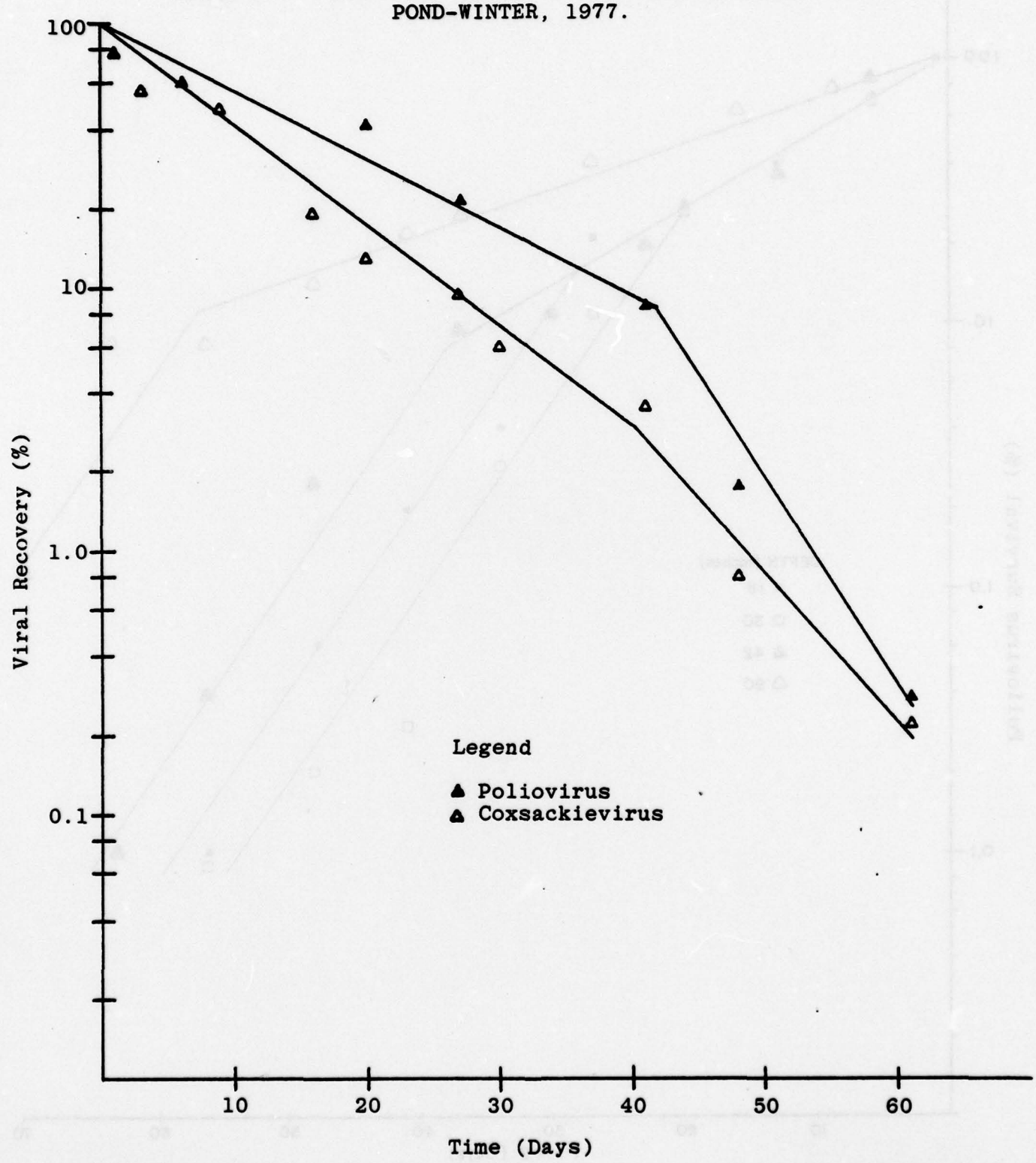
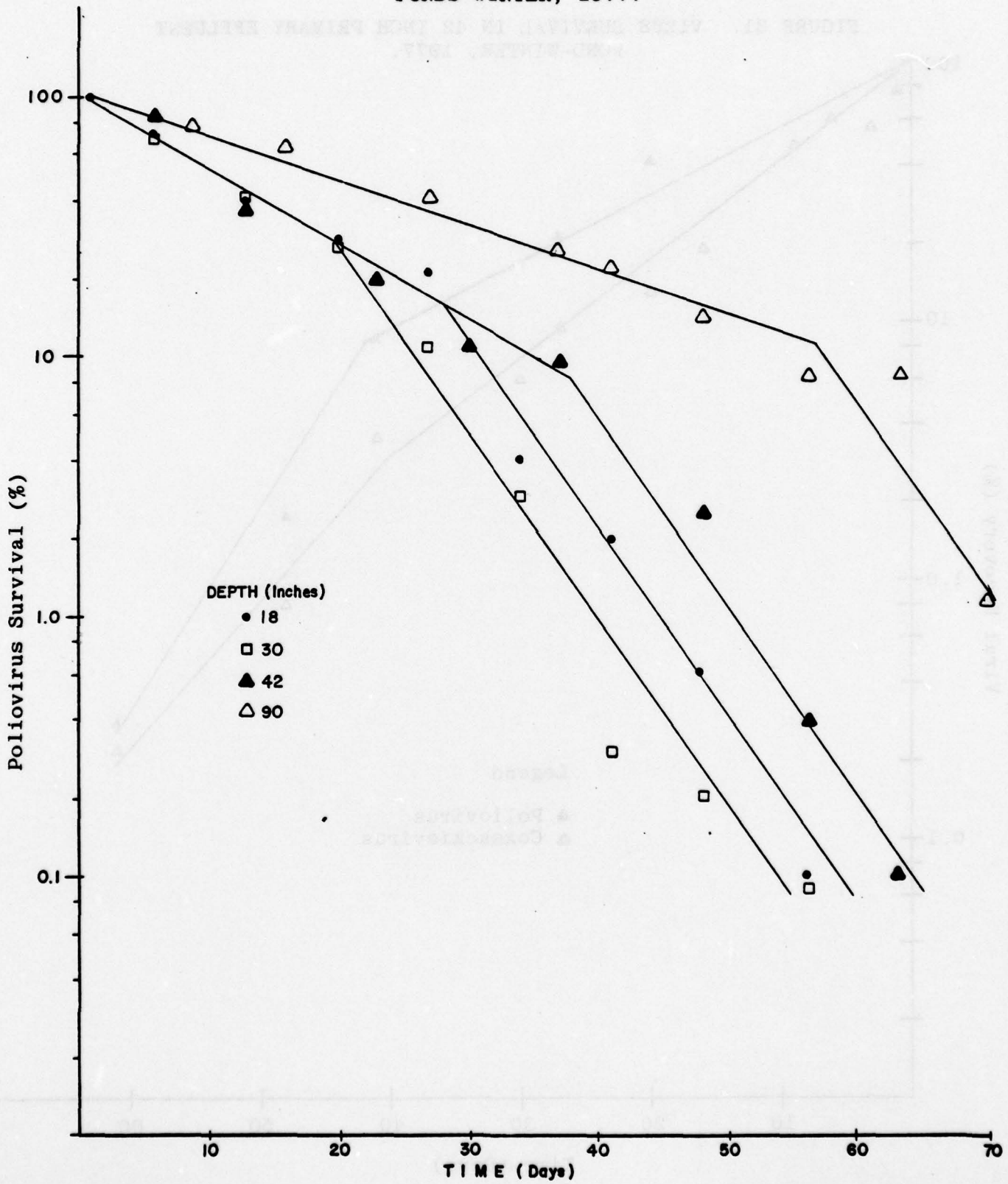


FIGURE 32. POLIOVIRUS SURVIVAL IN PRIMARY EFFLUENT PONDS-WINTER, 1977.



place. As mean temperature was similar in all four ponds (TABLE 29), this change in rate of inactivation was due to some alteration of the pond environment that took place at different times in ponds of different depths.

In all ponds, an elevation in pH closely followed this change in the rate of virus inactivation (TABLE 30). In all four ponds, the pH was uniformly low, between 7.2 and 7.5, in the shallow portion of the survival curves. At the point where the inactivation rate increased, there was an increase in pH to about 8.0. The elevation of pH, in turn, was due to the presence of algae in the ponds. We have shown above that algal photosynthesis increases the alkalinity of water. Apparently, in the first days of the field tests, the low concentration of algae (TABLE 31) in conjunction with the natural buffer capacity of the pond waters resulted in a stable pH. As settling occurred and the algae community became established, the buffering capability of the ponds became exhausted. One would expect that this would occur more rapidly in the shallower ponds, and this generally appeared to be the case.

Virus recovery from the sediments of the 42-inch final and primary ponds is illustrated in FIGURES 33 and 34. While the number of pfu in the water decreased shortly after addition of virus, the number of infectious virions in the settled solids increased dramatically, between 20- and 40-fold, during the first 6 days. For the next 5 weeks, the total pfu in the sediment of these ponds, which was about 10% of the total virus added to the ponds, remained constant.

Another biological variable that was monitored closely during this test was the fate of indigenous fecal coliforms in both the water and sediments of the primary effluent ponds. FIGURE 35 illustrates coliform survival in the liquid phase of the ponds. Over 99% of the organisms were lost within 20 days of residence time. This rate of die-away was consistent with data reported from operational waste stabilization ponds. It should be noted that the rate of coliform die-off was reduced in all ponds after about 18 days. This indigenous population of fecal coliforms was perhaps composed of a large number of organisms with different resistance to environmental stress. After the initial, rapid die-off of the less resistant organisms another group of more resistant forms persisted. This group was eventually affected by environmental stress or, possibly, factors which limited endogenous respiration. Another explanation for the results seen in FIGURE 35 is that the organisms may have begun to regrow after 15 days of detention. This would retard the rate of coliform decay.

The pattern of coliform reduction in the primary pond sediments differ from that of the overlying liquid (FIGURE 36). In the former case, die-away of the organisms was fairly gradual throughout the sampling period. However, reduction was more rapid in the shallower ponds as was the case in the water columns.

TABLE 29. TEMPERATURE (°C) OF PRIMARY EFFLUENT PONDS, WINTER, 1977.

Time (days)	Pond Depth (inches) 18	30	42	90
1	4	4	5	4
3	5	5	5	6
6	3	3	3	5
9	5	4	4	5
13	7	7	7	7
20	3	3	2	5
23	8.5	7	7	7
27	7	7	7	7
30	10	10	9	9
34	7	7	7	7
37	8	8	8	9
41	9.5	9.5	9	10
48	8	9	9	9
56	11	11	11	11
63			15	14
72			12	12

TABLE 30. pH OF PRIMARY EFFLUENT PONDS, WINTER, 1977.

Time (days)	Pond Depth (inches) 18	30	42	90
1	7.5	7.6	7.5	7.4
3	7.5	7.3	7.3	7.2
6	7.3	7.4	7.5	7.4
9	7.5	7.5	7.5	7.3
13	7.4	7.5	7.4	7.3
16	7.5	7.6	7.4	7.4
20	7.6	7.6	7.6	7.5
23	7.5	7.6	7.5	7.4
27	7.6	7.7	7.6	7.4
30	7.7	8.2	7.7	7.5
34	7.7	8.8	7.8	7.4
37	8.0	9.0	8.1	7.4
41	9.2	9.3	8.7	7.5
48	9.8	9.5	9.0	7.8
56	9.8	9.8	9.1	7.8
63			8.5	8.3
72			8.3	7.7

FIGURE 33. VIRAL RECOVERY FROM 42 INCH FINAL EFFLUENT POND SEDIMENT-WINTER, 1977.

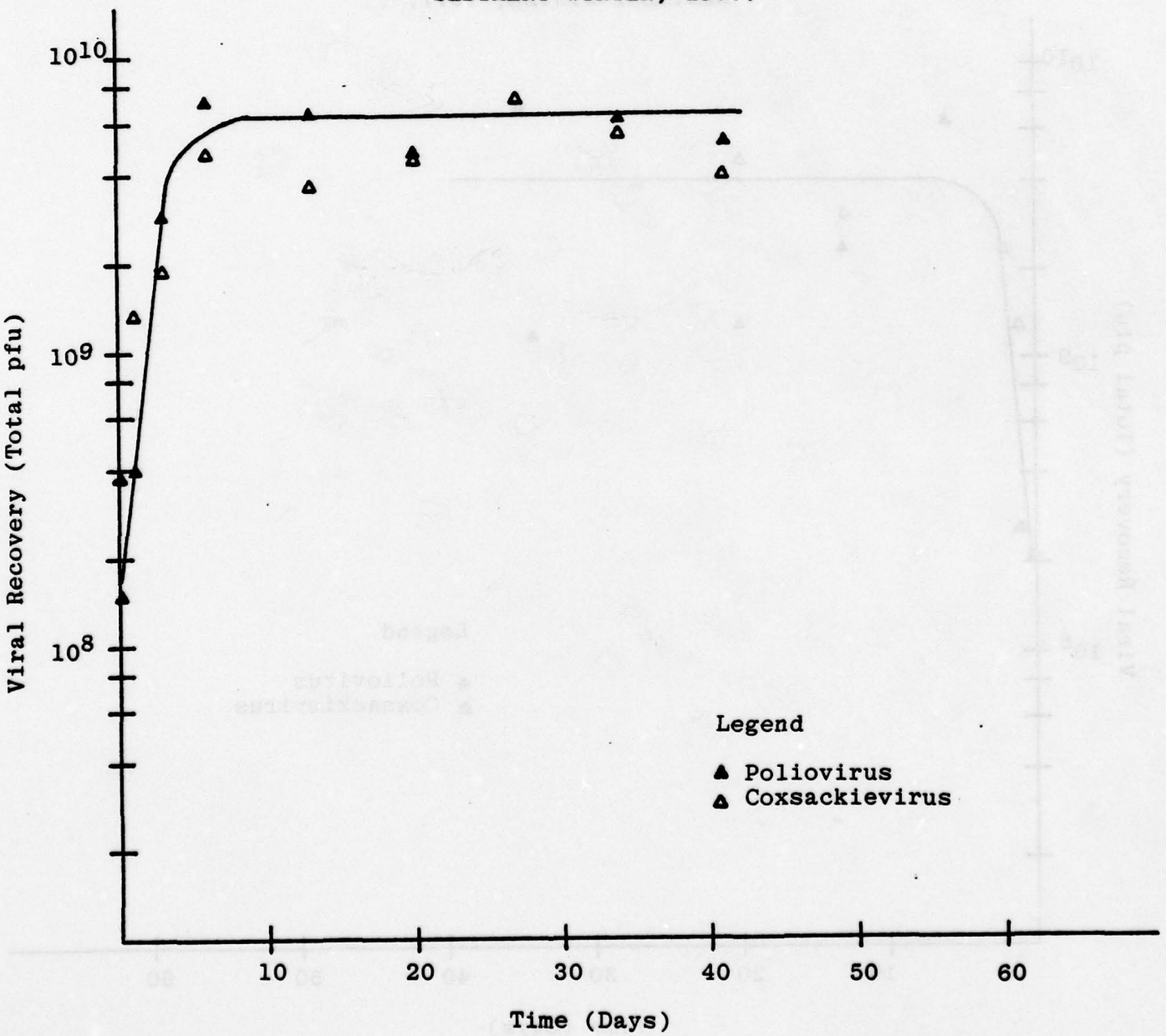


FIGURE 34. VIRAL RECOVERY FROM 42 INCH PRIMARY EFFLUENT POND SEDIMENT-WINTER, 1977.

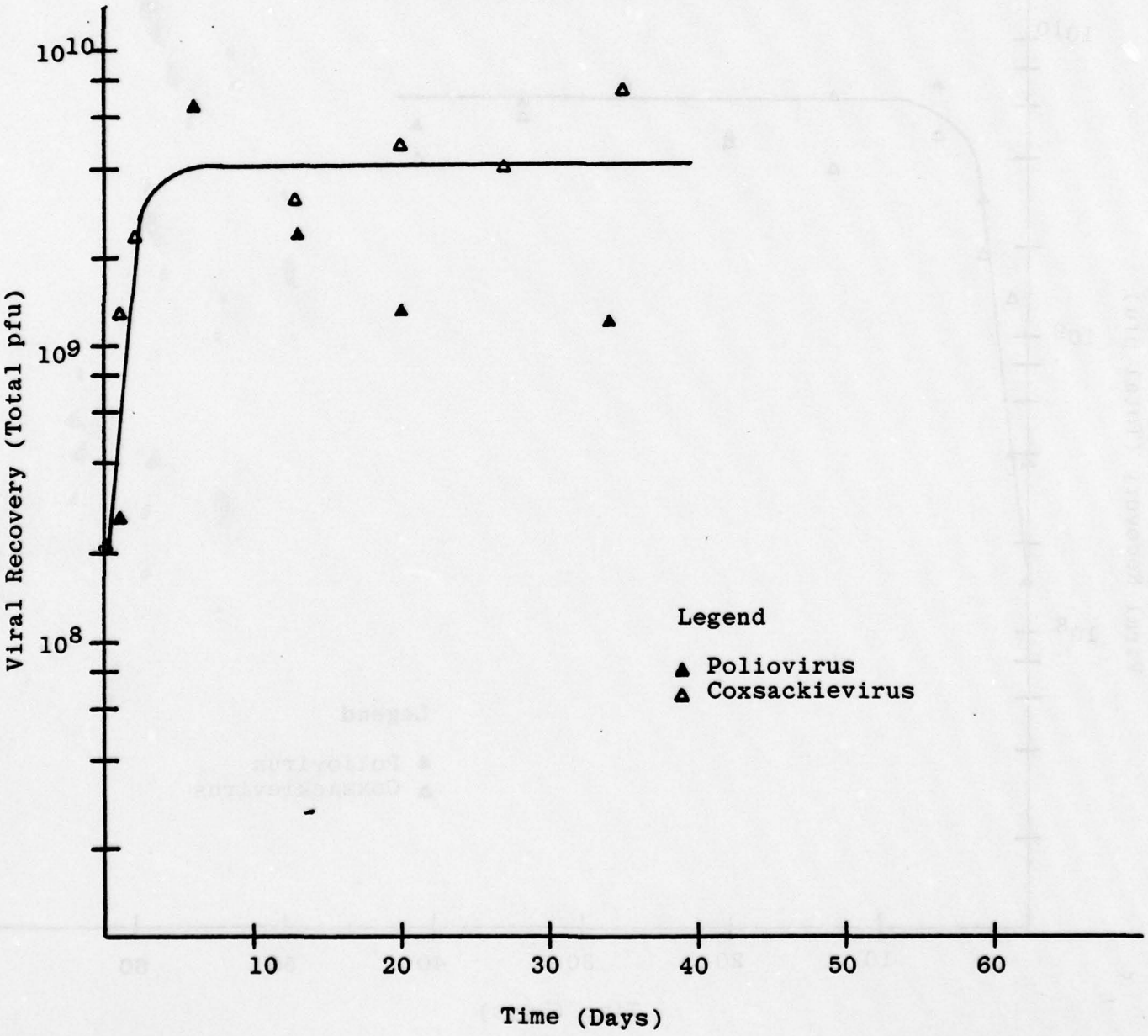


FIGURE 35. FECAL COLIFORM SURVIVAL IN PRIMARY EFFLUENT PONDS-WINTER, 1977.

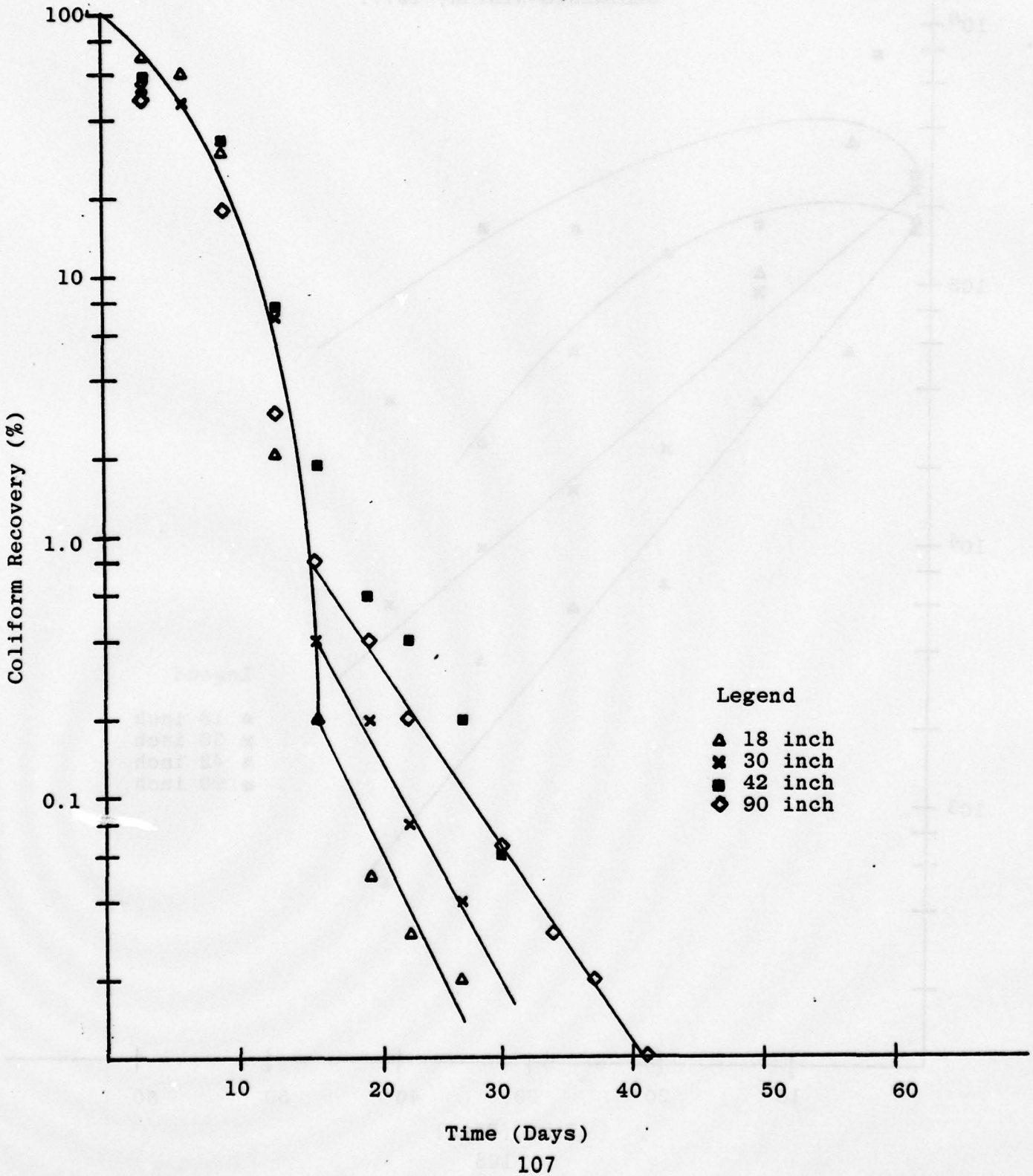


FIGURE 36. FECAL COLIFORM RECOVERY FROM PRIMARY EFFLUENT POND SEDIMENTS-WINTER, 1977.

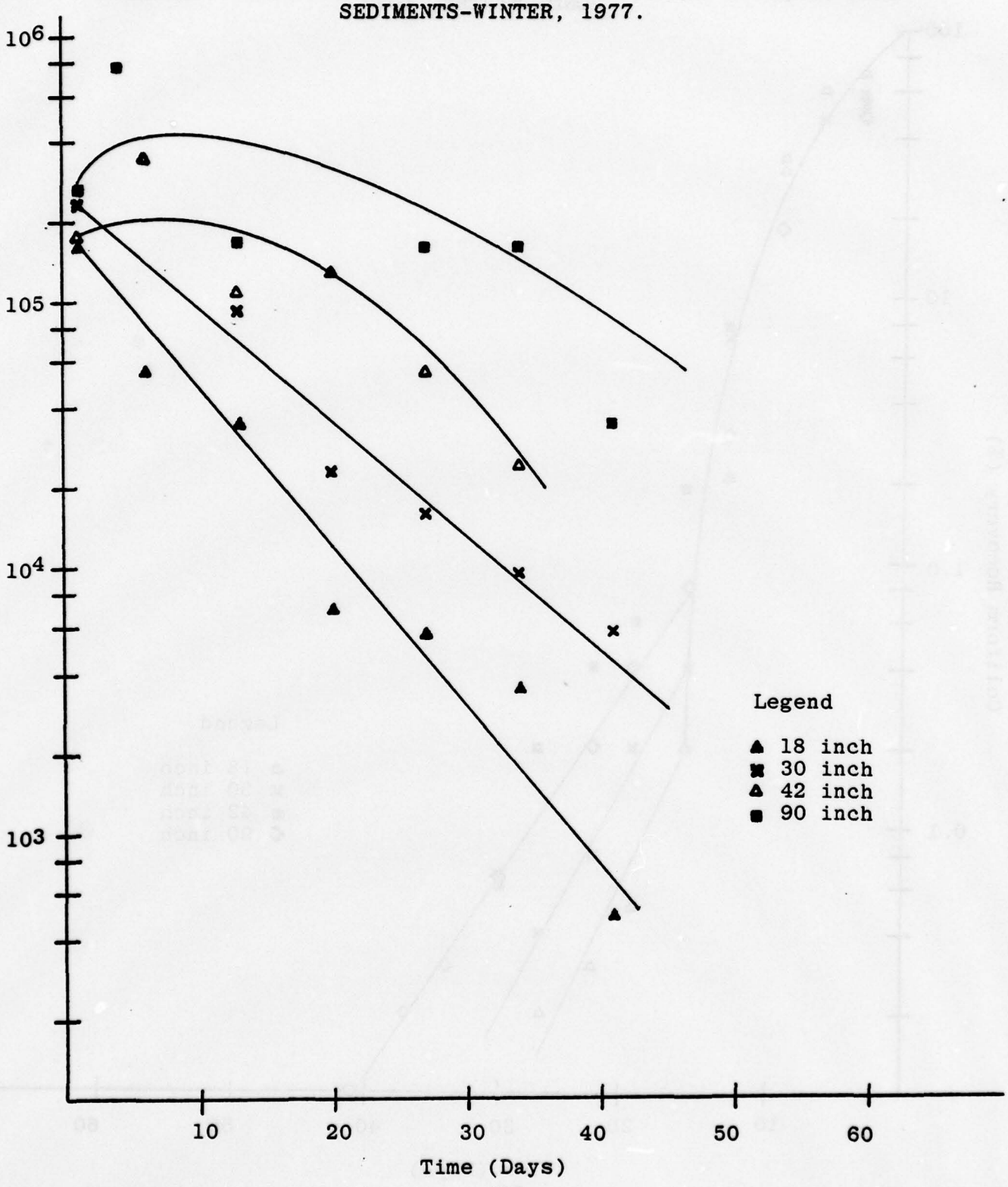


TABLE 31. CHLOROPHYLL CONCENTRATION (μg/l) IN PRIMARY EFFLUENT PONDS, WINTER, 1977.

Time (days)	Depth of Pond (inches)			
	18	30	42	90
1	70	160	150	190
6	40	80	140	250
13	0	90	110	140
20	0	50	80	100
27	0	80	80	100
34	40	160	180	90
48	2220	440	350	910
56	570	360	3760	870
63				310
72			720	2100

It is clear that the pattern of fecal coliform survival does not follow the reduction of poliovirus or Coxsackievirus in the holding ponds. Inactivation of viruses in the water column was slow until elevated pH increased the rate. Dieaway of coliforms was very rapid initially, but tapered off. Survival of virus in sediments was fairly constant during the test period. In contrast to this, fecal coliforms in the sediments experienced a constant reduction over this same time.

DATA ANALYSIS

Tests of hypotheses carried out in this analysis required that the data satisfy a specific set of assumptions. Two crucial assumptions are that the errors in the models (Equations 6 and 7) are normally distributed and are statistically independent. For these data, usually there were not enough observations to justify normality. As regards independence, efforts were made to reduce independence by introducing peritnent covariates in the model, but sometimes the autocorrelations were rather high. However, it has been assumed that these assumptions are satisfied. It is surprising, therefore, that the results arrived by this analysis support the results obtained in laboratory experiments. However,

it should be emphasized that the results of the analysis should be regarded as an indication of trend only, and should not be relied upon heavily.

Evaluation of Sampling

Since the inactivation rate of poliovirus may differ in the regions (depths) sampled in the model holding ponds, an attempt was made to determine whether the survival models were significantly different among these regions. The results of these comparisons for the Spring, 1976 and the Winter, 1977 field tests are presented in TABLES B-1 and B-2, APPENDIX B. At the .05 level of significance the survival models do not differ within ponds with the exception of the middle and bottom of the 90-inch final effluent pond during Spring, 1976 in which the differences are slight. It should be noted that the comparisons in TABLE B-1(a) are based on small sample sizes and should be regarded as indications of trends.

In view of these results, the observations at different levels within each pond were combined to give larger sets of data. The total number of observations within each pond after combining are presented in TABLE 32.

TABLE 32. NUMBER OF OBSERVATIONS AVAILABLE FOR DATA COLLECTED AT VARIOUS DEPTHS WITHIN PONDS.

Test Series	Pond Depth (inches)			
	18	30	42	90
Final Effluent				
Fall, 1975	16	16	16	18
Spring, 1976	8	11	8	12
Summer, 1976	6	10	10	15
Winter, 1976	18	20	18	35
Winter, 1977	14	26	26	28
Primary Effluent				
Spring, 1976	--	30	--	33
Summer, 1976	18	16	15	24
Winter, 1977	32	30	32	50

Model Estimation

TABLES B-3(a) and B-4(a), APPENDIX B, include poliovirus survival models for the ponds during all seasons for final and primary effluent, respectively, when all the data are considered. A linear model in pH and time seems to be adequate. It does not appear that pH is an important factor in Spring, 1976 for either wastewater. Such a model is not adequate, however, for the 30-inch final effluent pond in Spring and Summer, 1976.

As the plots of the total available number of observations deviated significantly from a first order relationship, an attempt was made to estimate the models from data which had been truncated. It was observed repeatedly that the data deviated most frequently when there was a significant increase in pH (generally above 8.0). This observation coupled with the results of the laboratory studies on the effects of test virus removal as a function of pH became the basis for selecting the truncation point. TABLE 33 presents the total number of observations available for analysis after truncation of the data along with the truncation point selected (time in days).

TABLE 33. NUMBER OF OBSERVATIONS AVAILABLE FOR DATA (TRUNCATION POINT IN DAYS IS SHOWN IN PARENTHESIS).

Test Series	Pond Depth (inches) 18	30	42	90
Final Effluent				
Fall, 1975	10(5.0)	10(5.0)	10(5.0)	12(5.0)
Spring, 1976	6(7.0)	6(10.0)	8(6.0)	9(10.0)
Summer, 1976	4(2.0)	6(4.0)	8(6.0)	12(6.0)
Winter, 1976	16(21.0)	18(21.0)	16(21.0)	27(25.0)
Winter, 1977	10(10.0)	16(25.0	18(25.0)	--
Primary Effluent				
Spring, 1976	--	18(45.0)	--	27(45.0)
Summer, 1977	10(8.0)	14(15.0)	13(15.0)	21(15.0)
Winter, 1977	30(42.0)	26(42.0)	28(42.0)	48(60.0)

TABLES B-3(b) and B-4(b), APPENDIX B, present models for final and primary effluent, respectively, for the truncated data. Except for the 90-inch final effluent pond during Winter, 1976 and Winter, 1977 and the 30- and 90-inch primary effluent ponds in Summer, 1976, linear models in pH and time are adequate for the truncated data. With the truncated data, pH tends to be significant explanatory variable less often than when the whole data are considered. With the exception of the 18- and 90-final effluent ponds during Spring and Summer, 1976 respectively, time remains a significant factor in poliovirus survival.

TABLES B-3(c) and B-4(c), APPENDIX B, present survival models in terms of pH, time and temperature for the final and primary effluents, respectively, when all the data are considered. TABLES B-3(d) and B-4(d), APPENDIX B, present similar models for the truncated data. With few exceptions temperature is not a significant explanatory variable in these models.

Comparison of Models

Ponds Within Seasons (Final Effluent).

TABLE B-5(a) presents overall and pairwise comparisons of models in terms of time and pH for the final effluent ponds in each season. TABLE B-5(b) presents a similar comparison when temperature also is included. The survival models in terms of time and pH for 18-, 30-and 42-inch ponds in Fall, 1975 do not differ significantly. However, the models for all three of these ponds are significantly different from that of the 90-inch pond. The results of the above comparisons [see TABLE B-5(b)] remain the same when temperature is added to the model.

The results of the comparisons between Spring and Summer, 1976 are indicators of trends of virus behavior among the ponds as sample sizes are too small for the analysis to be completely reliable. However, the results seem to indicate that the models of each pond are different for Spring, 1976, and for the comparisons that were possible for Summer, 1976. For Winter, 1976 with and without temperature the models for each pond within each possible pair are significantly different. However, the survival models for the 18-, 30-and 42-inch ponds do not differ significantly during Winter, 1977.

Seasons Within each Pond (Final Effluent)

TABLE B-6(a), APPENDIX B, presents overall and pairwise comparisons of seasons with each pond when time and pH are considered. TABLE B-6(b), APPENDIX B, presents similar comparisons when temperature is also considered. This provides partial answers to the question regarding the differences in models from season to season. It can be seen that within the 18-inch pond there are different survival models between each season tested. For the 30-inch pond, the models for every pair of seasons except Spring, 1976, Winter, 1977

were significantly different. Similar results were also noted in the 42- and 90-inch ponds. Whenever the comparison was possible with the temperature data, all the possible pairs of seasons for 18-, 30- and 90-inch ponds have significantly different models. For the 42-inch pond the models for all the pairs are significantly different except the pair Winter, 1976, Winter, 1977 which differ marginally. Thus, it seems that generally for the same depth of ponds, seasons do have different survival models.

Ponds Within each Season (Primary Effluent)

TABLE B-7(a), APPENDIX B, presents comparison of models for each pair of ponds within seasons for the primary effluent when time and pH are considered. TABLE B-7(b), APPENDIX B, presents a similar comparison when temperature is included. It can be seen that with the exception of the pairs 18, 30; 18, 90; 30, 42 and 42, 90 for Summer, 1976 and 18, 30 in Winter, 1977, all other pairs in each season have significantly different models in terms of time and pH. When temperature is included all the pairs except 18, 30 have significantly different models during Winter, 1977. This is the only season when temperature data are available on primary effluent. However, the results of comparison for Winter, 1977, with or without temperature, remain the same. Although there is no definite pattern for Summer, 1976, it can be concluded that the two shallow ponds tend to have similar models for the primary effluent for Winter, 1977.

Seasons Within each Pond (Primary Effluent)

Survival models differ significantly when seasons are compared within each primary effluent pond (TABLE B-8, APPENDIX B). As can be seen, essentially all values P are essentially small.

Pondwise Comparison of Final and Primary Effluents

TABLES B-9(a) and B-9(b), APPENDIX B, present pondwise comparison of the models for the final and primary effluents without and with temperature, respectively. During both Winters survival in the 42-inch final and primary effluent ponds are different. During the same test series, however, when shallow ponds of the same depth are compared there appears to be no difference in the survival models.

CONCLUSIONS

From the available published literature on ponds as a wastewater treatment link, it appears that multiple ponds in series are superior to single ponds in the removal of BOD from domestic wastewaters. This is true even if the surface area of a single pond is greater than that of the summed area of the multiple ponds. Parker (1950), reporting on BOD removal in four lagoons in series, found that more than half the removal occurred in the first day, an additional fourth in the next four days, and less than 10% more in the next

five days. Thereafter, he saw little change in BOD. This is consistent with data reported by Oswald (1963), assigning 90% of the physical sedimentation observed in ponds to the first three days, and with those of Meron (1961) who reported the greatest BOD reduction was nearest to the influent pond zone.

These data are supported by the experimental data reported here, which show that those viruses which become deposited in the sediments do so within the first several days in the pond.

Young (1974) has pointed out that there is no predictive design information on pathogen removal. Such data as exist are a by-product of designs for organics removal. Design criteria for pathogen removal is a critical need in view of the 1973 EPA regulations on coliform discharge limitations. The literature on coliform removal by ponding indicates that survival of coliforms (and, presumably, of bacterial pathogens as a group) is dependent on a number of factors. These include environmental variables such as temperature, ultraviolent penetration, light penetration and ice cover. In addition, construction and operation factors such as total loading, detention time in the first pond in a series, pond depth-to-surface ratios all influence E. coli survival. Franzmathes (1970) reported the number of ponds in the series to be significant in E. coli removal (perhaps as in BOD removal). Although Parker (1962) and Hodgson(1964) noted the increase in algal population in later ponds in a series, the relationship of algal density to coliform removal is not clear.

Even less has been reported on the fate of enteric viruses in working oxidation or holding ponds. Malherbe and Strickland-Chomley (1967) recovered viruses for as long as 56 days after seeding in a pond facility with 38 days theoretical detention time. Shuval (1969), using a four-pond series, reported that he obtained no more than 67.5% removal of enteric viruses with 20 days detention. These observations should be evaluated in light of the results obtained from the model pond studies reported here. Certainly, depth is a significant factor in virus removal, especially in those seasons where temperature stratification occurs. Temperature, too, is important. Viral deposition with sediments (not studied in earlier reports on ponds) plays a significant role in enterovirus removal and survival. Finally, the role of algae appears significant, at least indirectly, in effecting marked pH changes in the ponds, changes which increase poliovirus inactivation rates significantly. Changes in pH can be affected by season, pond depth, loading rate, pond number (and sequence) in a series, as well as by diurnal light patterns (Neel et al., 1961).

Despite the limitations on the sample sizes (number of observations involved) statistical evaluation of the model pond data has led to the following conclusions:

1. It was observed that the linear model for z in terms of pH and time was adequate for describing the relationship between poliovirus survival and the parameters evaluated.

2. Temperature as an explanatory variable did not seem to influence virus survival during any test series, probably because of its narrow range. For the truncated data, the range of pH also became narrower, and thus, became somewhat less significant.

3. For the final effluent (with the exception of Winter, 1977) within each season, shallower ponds tended to have significantly different models than did the deeper ponds. A similar statement holds true for the primary effluent (except for Summer, 1976) in which case no specific trend was noted.

4. For a fixed depth of pond, different seasons generally were found to result in significantly different survival models. It was interesting to observe that for fixed depths, the two winters (Winter, 1976 and Winter, 1977) resulted in different models. This was probably due to the fact that the two winters were dramatically different in their severity.

5. Both final and primary effluents were observed to have different survival models for the ponds in warm weather. For colder weathers, however, shallower ponds behaved similarly for both of the test wastewaters, whereas, deeper ponds behaved differently.

PRELIMINARY DESIGN CRITERIA

Conventional design criteria for ponds involve such parameters as depth, surface area, detention time and organic loading. Not all of these parameters are independent; therefore, different regulatory agencies and design engineers will use but two or three of these parameters. Although detention time is used as a design criterion to determine pond area in some circumstances, organic loading is considered to be the best criterion (ASCE, 1959).

Other considerations to be used in pond designing include rainfall, relative amount of sunshine, temperature, direction of prevailing winds, soil structure and the proximity of the ponds to populated areas. In most cases, however, these factors are secondary.

ORGANIC LOADING

Canter and Englande (1970) conducted a survey of pond practices throughout the United States. For purposes of comparison they divided the nation into three geographical regions based on latitude: those states above 42° latitude, those between 37° and 42° and states below 37° latitude. The results of their survey on organic loading is presented in TABLE 34. It can be seen that mean loading rate decreases in the more northerly sections of the nation. The trend of decreasing organic loading at higher latitudes is clear from other surveys. The American Society of Civil Engineers (1959) suggests a loading of between 15 and 20 lb. BOD/acre/day for cold climates and between 50 and 150 lb. BOD/acre/day for warm climates. Davis (1963) stated that most ponds in Texas operate at 50 lb. BOD/acre/day. According to Svore (1968) the average design criteria in the South and Southwest call for up to 50 lb. BOD/acre/

TABLE 34. ORGANIC LOADING IN PONDS IN THE UNITED STATES. (after Canter and Englande, 1970)

	Above 42°	37°-42°	Below 37°
Number of states	18	17	15
BOD Loading (lb/acre/day)			
Mean	26	33	44
Range	16.7-40	17.4-80	30-50
Median	21	33	50
Loading (population/acre)			
Mean	124	189	267
Range	100-200	100-400	175-300
Median	100	200	295

TABLE 35. DETENTION TIME (DAYS) IN PONDS IN THE UNITED STATES. (after Canter and Englande, 1970)

	Above 42°	37°-42°	Below 37°
Mean	117	82	31
Range	30-180	25-180	20-45
Median	125	65	31

day with trends toward the higher figures. In Missouri single ponds may be loaded at 45 lb./acre/day while 2-celled ponds may receive 60 lb./acre/day in the first cell (Decker, 1963). Design criteria for Tennessee call for maximal organic loadings of 200 persons/acre (Fleming, 1963). Van Heuvelen et al. (1960) state that BOD loadings from 10 to 34 lb./acre/day are used throughout the Missouri Basin States. Standards set by the Great Lakes-Upper Mississippi River Board of Sanitary Engineers for the states of Illinois, Pennsylvania, Indiana, Iowa, Michigan, Minnesota, Missouri, New York, Ohio and Wisconsin call for 16.7 lb. of BOD/acre/day, 100 persons/acre and 10,000 gal/acre/day (Barnes, 1963). Barnes also listed the recommended loadings in lb. BOD/acre/day by other northern states (i.e., North Dakota, 20 to 40; Minnesota, 15 to 25; South Dakota, 11 to 25; Colorado, 100 to 200 persons/acre; Wyoming, 35 lb/acre/day).

DETENTION TIME

The survey of Canter and Englande (1970) included detention times used in waste stabilization ponds throughout the U.S., also (TABLE 35). It can be seen that detention time increases in the more northerly sections of the country. The American Society of Civil Engineers (1959) recommends a detention period of 30 days for ponds in both cold and warm climates. In Texas ponds 30 days of detention time are normally used (Davis, 1963). In the Missouri Basin flow through ponds have a minimum of 60 days detention with 90 to 120 days often specified (Van Heuvelen et al., 1960). According to Canter and Englande (1970), some northern states recommend retention of the entire winter flow because of treatment difficulties due to ice cover.

DEPTH

The average recommended liquid depth throughout the U.S. is four feet. Minimal depths are specified to discourage weeds. Values greater than the maximal recommended depth result in poor treatment. Minimal depth above 42° latitude is two feet (Minnesota, New York, Vermont, Michigan and Alaska), the maximal depth is six feet (Michigan). In Idaho, the recommended operating depth in summer is two to three feet and four to five feet during the winter. Between 37° and 42° the minimal recommended depth is two feet (Delaware, Indiana, Nebraska, Ohio and Pennsylvania), maximal is 15 feet (Delaware). In Iowa the recommended depth is four feet for ponds one acre or less and five feet for ponds larger than one acre. In Missouri, depths of two to three feet for ponds less than or equal to ten acres and two to five feet for ponds greater than ten acres are recommended. Below 37° latitude the minimal recommended depth is three feet (Alabama, Arizona, Georgia, Hawaii, Mississippi, North Carolina, Oklahoma, South Carolina and Texas) and the maximal is five feet (Alabama, Arizona, Arkansas, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee and Texas) (Canter and Englande, 1970). The American Society of Civil Engineers (1959) suggests a pond design depth of five feet or less for cold climates and between two and four feet for warm climates.

POND CONSTRUCTION

Multiple cells are recommended by most states. Single ponds are suggested for use only in small installations. Single ponds must be less than one acre in Colorado and Oregon, two acres in Nebraska, six acres in Kentucky and New York, six to eight acres in Idaho and ten acres in Alabama. Kansas indicates that single ponds should serve less than 25 population equivalents. Single ponds in Pennsylvania are to be used only for primary or intermediate treatment. In Missouri, effluent from single ponds must be discharged into flowing streams. The states of Delaware, New Hampshire, North Dakota, South Dakota, Texas, Utah and Wisconsin do not recommend the use of single ponds. According to Svore (1968), it is current practice in the Midwest and Southwest to use two ponds in series. Recommended loadings in the first pond are 20 to 50 lb/acre/day. The second receives about the same loading rate, but since this pond is receiving treated effluent it is much smaller in size. The Missouri Basin Engineering Health Council recommends the use of serial ponds when low concentrations of algae in the effluent are desirable. Cells may be operated in parallel during Fall, Winter and early Spring when algae development is less intensive and in series during the Summer. Series operation of ponds is also beneficial when high levels of BOD or coliform reduction are required (Van Heuvlen, 1960).

Canter and Englande (1970) indicate that all states recommend submerged inlets far enough from the nearest bank to prevent circulation interference. For square or circular ponds, 32 states recommend center discharge, for larger or rectangular lagoons, 17 states recommend discharge at the center point most distant from the outlet. The Missouri Basin Engineering Health Council recommends discharge of influent more than 200 feet from the nearest bank in medium sized ponds and 400 feet from the bank in ponds larger than 40 acres (Van Heuvelen et al., 1960). The preferred outlet location in most states is generally at a point farthest from the inlet on the windward side to minimize short circuiting.

DESIGN CRITERIA

A major objective of this study was the identification of significant design criteria for ponds, criteria which will be subject to further verification by field study. These preliminary criteria were to be based on existing ones but include modifications which would provide for optimized reduction of viruses. The modifications were to be based on the review of existing literature and on the results of the field and laboratory studies reported as a part of this research.

The results of these studies (review of the literature, field and laboratory studies) indicate that factors such as detention time, temperature, pH and depth of the ponds have a significant impact on virus survival in the model ponds and, where evaluated, in the laboratory studies. Light was observed to have a lesser impact on test virus survival: dissolved oxygen and algae,

per se, a nondistinguishable impact. TABLE 36 contains a ranking of the parameters observed in this study, and the relative influence these parameters have on virus inactivation in ponds. The ranking has been influenced to some extent by sound engineering, practice used in the design of ponds for BOD removal.

TABLE 37 contains an assimilation of the design criteria discussed previously and the parameters observed in this study to influence virus inactivation (TABLE 36) into preliminary design criteria for holding ponds to be used prior to land application of wastewaters which have undergone primary or secondary (biological) treatment. Inherent in these criteria are three assumptions: the ponds are facultative, ponds exist in series and they employ baffles or multiple inlets to distribute the load over a wide area. Where primary effluent is involved, larger holding ponds are required to obtain the same results.

As can be seen in TABLES 36 and 37, it should be possible to develop a model for each region of the country where only the coefficients would be different. Such a model could take the form:

$$Z = \alpha o + \alpha_1 D_T + \alpha_2 D + \alpha_3 L + \alpha_4 T + \alpha_5 P + \alpha_6 \ell + \varepsilon$$

where: $Z = \ell n\ (y+1)$ (y is the concentration of virus remaining)

D_T = detention time

D = depth

L = BOD loading (lb/acre/day)

T = temperature

P = pH

ℓ = solar intensity

ε = error term

Development of such a model would require the collection of field data from similar facilities operating in several regions of the country. These field studies must focus on functioning multicell systems where access to multiple sampling sites can be planned to yield statistically validated answers. Such studies should use sampling sites with different depths in each cell in the series, they should collect sediments from each cell's influent area and deepest point, they should be carried on through at least two years in all seasons, they should correlate BOD loading and other chemical and physical characteristics with coliform, enterovirus, coliphage, and selected pathogen levels as well as algal population fluctuations. It would be appropriate for such studies to be carried out at several sites differing radically in seasonal temperature extremes.

TABLE 36. RANKING OF PARAMETERS OBSERVED TO INFLUENCE VIRUS INACTIVATION IN PONDS.

Parameter	Rank	Remarks
Detention Time	1	As with most biological processes, time results in decreased viral levels
pH	2	Highly significant with major deviation from neutrality
Temperature	2	High temperatures promote inactivation; low temperatures retard inactivation
Wastewater	3	Virus survival in primary effluent exceeded survival in final effluent
Depth	3	Shallow ponds (1.5-3.5 ft) promote inactivation; deep ponds (7.5 ft) retard inactivation
Solids	4	Virus adsorbed to deposited solids survive for extended periods
Light	5	Light can have some impact
Dissolved Oxygen	6	Does not significantly affect virus survival rates

TABLE 37. PRELIMINARY POND DESIGN CRITERIA FOR MAXIMIZING VIRUS REDUCTION.

Design Parameter	Value	Remarks
Detention Time	30 days	For cold climates (below 10C), detention should exceed 30 days
Depth	1.5-3.5 ft	For cold climates, additional depth (1-2 ft) should be provided for periods with significant ice cover
Discharge Point	Near Surface	Required to prevent solids carryover for irrigation.

APPENDIX A

COLIFORM REDUCTION IN WASTE STABILIZATION PONDS RECEIVING RAW WASTEWATER

APPENDIX A

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

	Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type+	Comments	Reference
MIDWEST	W	NG	50	NG	85.9	T	Single pond	Geldreich et al., 1964
	S	NG	50	NG	94.4	T	Single pond	
	NG	NG	50	NG	87.9	F	Single pond	
	All year	NG	6 to 23	NG	>95	F	Average for 5 towns, 4 using 1 pond, 1 using 3 in series	Towne et al., 1957
	All year	NG	91	NG	95	T	Single pond	Neel and Hopkins, 1956
	S	87	20	1.7×10^4	99.995	T		Neel et al., 1961
	F	87	20	1.2×10^4	99.973	T	Seasonal averages for single pond	
	W	87	20	5.0×10^3	99.910	T		
	Sp	87	20	1.3×10^4	99.964	T		
	S	44	41	2.2×10^4	99.937	T		
	F	44	41	2.0×10^4	99.955	T	Seasonal averages for single pond	
	W	44	41	5.0×10^3	99.991	T		
	Sp	44	41	1.3×10^4	99.965	T		

*NG - information not given
+T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type+	Comments	Reference
S	29	61	2.8×10^4	99.92	T	Seasonal averages for single pond	
F	29	61	1.5×10^4	99.967	T		
W	29	61	2.5×10^4	99.955	T		
S	29	61	4.1×10^4	99.889	T		
S	22	81	3.2×10^4	99.909	T	Seasonal averages for 1 pond	
F	22	81	1.2×10^4	99.973	T		
W	22	81	3.5×10^4	99.936	T		
Sp	22	81	4.8×10^4	99.870	T		
S	17	101	4.3×10^4	99.877	T	Seasonal averages for 1 pond	
F	17	101	1.4×10^4	99.968	T		
W	17	101	8.1×10^3	99.985	T		
Sp	17	101	1.8×10^5	99.514	T		
All year	NG	120	3.1×10^4	99.973	T	Single pond	Claire et al., 1961

*NG - information not given
+T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type+	Comments	Reference
All year	NG	100	3.3×10^4	99.978	T	In series overall reduction 99.999%	
All year			6.0×10^3	88.818	T		
All year	NG	60	2.2×10^4	99.985	T	Depth 5'	
All year	NG	60	1.7×10^4	99.988	T	Depth 2.5'	
All year	45	49 to 82	3.0×10^5	90.910	T	Single pond	Horning et al., 1964
All year	45	49 to 82	6.0×10^4	93.333	F		
All year	15	117 to 194	6.0×10^5	81.819	T	Single pond	Neel et al., 1961
All year	15	117 to 194	1.7×10^5	81.111	F		
All year	1.8 to 3.3	20 to 135	1×10^5	50	T	Single pond receiving secondary effluent	Loehr and Stephanson, 1965
W	NG	22.9	1×10^3 to 2.4×10^4	>99.9	T	1 pond, 4 seasons	Towne et al., 1957
Sp	NG	22.9	1.2 to 2.3×10^5	99.5 to 99.8	T		
S	NG	22.9	4.3×10^4 to 2.4×10^5	99.6 to 99.9	T		
F	NG	22.9	4.3×10^5	99.2	T		

*NG - information not given
T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type†	Comments	Reference
W	NG	7	4.2×10^{5} to 2.2×10^{6}	83.6 to 96.8	T	1 pond, 4 seasons	
Sp	NG	7	4×10^{3} to 1.3×10^{4}	99.6 to 99.9	T		
S	NG	7	4.0×10^{4} to 3.4×10^{5}	99.4 to 99.9	T		
F	NG	7	2.4×10^{5} to 4.6×10^{5}	99.1 to 99.6	T		
W	NG	6.8	2.0×10^{4} to 4.6×10^{4}	99.7 to 99.8	T	1 pond, 4 seasons	
Sp	NG	6.8	2.1×10^{4} to 4.3×10^{4}	97.3 to 99.1	T		
S	NG	6.8	1.5×10^{4} to 9.3×10^{4}	99.8 to 99.9	T		
F	NG	6.8	4.3×10^{4} to 9.3×10^{5}	59.5 to 98.1	T		

*NG - information not given
†T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type+	Comments	Reference
W	NG	9.4	5.8×10^5 to 1.3×10^6	95.9 to 97.4	T	3 in series	Towne et al., 1957
Sp	NG	9.4	1.9×10^4 to 2.7×10^4	98.8 to 99.2	T	3 in series; same series, 4 seasons	
S	NG	9.4	8.0×10^4 to 3.0×10^5	99.1 to 99.7	T	3 in series	
F	NG	9.4	2.3×10^4 to 4.8×10^4	95.4 to 97.4	T	3 in series	
W	NG	13.0	1.4×10^5 to 3.4×10^6	97.8 to 99.2	T	1 pond, 4 seasons	
Sp	NG	13.0	4.5×10^4 to 2.5×10	98.6 to 99.8	T		
S	NG	13.0	2.3×10^5 to 1.7×10^6	95.9 to 99.6	T		
W	NG	NG	NG	86	T	Single pond	Svore, 1968
F	NG	NG	NG	94	T		
NG	NG	NG	NG	88 to 98	F		

*NG - information not given
+T - Total
F - Fecal

APPENDIX A (*continued*)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

	Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type*	Comments	Reference
ARID SOUTHWEST	All year	7	590	3.0×10^5	51	T	Series	Merz et al., 1957
		1.1	820	1.2×10^5	80	T	Below are 2 ponds in series sampled at 6 different times	
	All year	30	109	1.5×10^5	79	T	Series	
		4.5	169	8.8×10^5	88	T		
	All year	43	66	8.3×10^5	76	T	Series	
		6.7	95	4.2×10^5	88	T		
	All year	39	89	1.3×10^6	81	T	Series	
		4.5	121	5.4×10^5	92	T		
	All year	9	280	3.2×10^6	29	T	Series	
		1.3	NG	1.5×10^6	67	T		
	All year	17	223	1.3×10^6	91	T	Series	
		2.3	NG	3.0×10^6	80	T		
	SE	30	NG	4.5×10^2	99.996	T	Single pond receiving secondary effluent	Warrington, 1952

*NG - information not given
†T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

	Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type*	Comments	Reference
SUB-TROPICAL COASTAL	W	NG	16 to 41	1.1×10^6	97.442	T	Single pond	Franzmanthes, 1970
	W	NG	16 to 41	5.6×10^4	98.0	F	Single pond	
	W	NG	31	7.1×10^4	NG	F	Single pond	
	W	NG	26	2.8×10^5	NG	F	Single pond	
FOREIGN	NG	NG	NG	1.1×10^7	NG	T	Average of 4 pond systems	Kott, 1973
	NG	NG	NG	1.3×10^6	NG	F	Average of 4 pond systems	
	Sp, Su	7	300	4.7×10^3	99.87	T	3 ponds in series	Johsi et al., 1965
	Sp, Su	7	300	1.4×10^3	99.94	F	3 ponds in series	
	Sp, Su	12.3	100	3.0×10^6	93.0	T	2 ponds in series	
	Sp, Su	12.3	100	1.4×10^6	93.0	F	2 ponds in series	
	S	3.5	NG	3.5×10^6	72	F	2 ponds in series with a third total reduction 96.75% (same system 2 seasons)	
		10.5	NG	3.9×10^5	90	F	2 ponds in series with a third total reduction 96.75% (same system 2 seasons)	
	W	3.5	NG	5.3×10^6	67	F	2 ponds in series with a third total reduction 97.56% (same system 2 seasons)	
		10.5	NG	8.6×10^3	99	F	2 ponds in series with a third total reduction 97.56% (same system 2 seasons)	

*NG - information not given
†T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type*	Comments		Reference
S	7	NG	1.4×10^7	86	F	2 ponds in series with a third total reduction 99.972%	same system 2 seasons	
	17.5	NG	3.1×10^5	94	F			
W	7	NG	8.9×10^6	45	F	2 ponds in series with a third total reduction 97.97%		
	17.5	NG	3.6×10^5	96	F			
S	3.8	NG	4.0×10^7	33	F			Parker, 1962
S	8.0	NG	4.0×10^6	90	F			
S	13	NG	2.1×10^6	50	F			
S	18	NG	6.6×10^5	66	F	8 ponds in series, total reduction 99.999%	same pond series, 2 different seasons (continued on next page)	
S	23	NG	2.5×10^4	96	F			
S	28.5	NG	1.5×10^3	94	F			
S	33.5	NG	1.1×10^2	94	F			
S	38.5	NG	1.3×10^1	88	F			

*NG - information not given
+T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type†	Comments	Reference
W	4.1	NG	1.9×10^7	66	F	6 ponds in series, total reduction 99.92%; same pond series, 2 different seasons	
W	8.6	NG	7.9×10^6	58	F		
W	14	NG	1.9×10^6	75	F		
W	19.2	NG	1.9×10^6	2	F		
W	24.6	NG	4.2×10^5	77	F		
W	30.5	NG	4.4×10^4	90	F		
NG	20	NG	6.4×10^3	97.5	F	2 ponds in series, total reduction 99.993%	Coetzee and Fourie, 1965
	15	NG	5.0×10^1	99.2	F		
NG	2.5	NG	1.6×10^3	81	F	4 ponds in series receiving secondary effluent, total reduction 98.7%	
	2.5	NG	7.5×10^2	52	F		
	2.5	NG	4.0×10^2	47	F		
	2.5	NG	1.1×10^2	53	F		
All year	38	270	NG	minimum 80 maximum 99	F	2 ponds in series	Hodgson, 1969
	61	NG	NG	66	F		

*NG - information not given
†T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type†	Comments		Reference
F	6.5	NG	1.9×10^7	93.45	T	One pond	2 ponds in series, total reduction 95.863%	Meron et al., 1965
	13.0	NG	1.02×10^7	46.32	T	Single pond total reduction 88.24%		
	19.5	NG	2.6×10^6	74.51	T			
	26.0	NG	1.2×10^6	53.85	T			
All year	NG	NG	2.5×10^7	68.25	F	2 in series, total reduction 99.956%		Parker et al., 1950
	NG	NG	3.5×10^4	99.86	F			
NG	NG	136	2.5×10^3	99.593	F	3 ponds in series receiving secondary effluent, total reduction 99.985%	same series different loadings	Gaillard and Crawford, 1964
		NG	6.4×10^1	97.44	F			
		NG	9.0×10^0	85.94	F			
NG	NG	170	4.8×10^2	99.922	F	3 ponds in series receiving secondary effluent, total reduction 99.998%		
		NG	1.3×10^2	73	F			
		NG	1.1×10^1	91.6	F			

*NG - information not given
†T - Total
F - Fecal

APPENDIX A (continued)

Coliform Reduction in Waste Stabilization Ponds Receiving Raw Wastewater

Season	Detention Time*	BOD loading lb/acre/day*	Organisms/100ml in effluent*	% Reduction	Coliform Type†	Comments	Reference
Sp	4	NG	NG	98.4	T	Same pond	Pahad and Pao, 1963
W	4	NG	NG	82.1	T		
S	2	NG	NG	98.6	T	Same pond	
W	4	NG	NG	79	T		
NG	21	NG	1	99	T	3 in series	Joshi et al., 1972

*NG - information not given
†T - Total
F - Fecal

APPENDIX B

TABULATED RESULTS OF STATISTICAL ANALYSIS

TABLE B-1. COMPARISON OF MODELS IN TERMS OF TIME AND pH FOR TOP AND BOTTOM OF ALL PONDS (ALSO MIDDLE FOR 90" PONDS) IN SPRING 1976.

Ponds	Sample Sizes	Depth	Equality of Intercepts *d.f.	F	**P	Equality of Slopes d.f.	F	P
			Final Effluent, Spring 1976					
18"	4,4	T,B	1,4	.115	.752	2,2	.132	.884
30"	6,5	T,B	1,7	.135	.592	2,5	.760	.515
42"	4,4	T,B	1,4	.067	.808	2,2	.260	.794
90"	3,4	T,M	1,3	.299	.628	2,1	.225	.229
90"	5,4	B,M	1,5	.084	.783	2,3	10.673	.043
90"	3,5	T,B	1,4	.287	.621	2,2	15,326	.061
			Final Effluent, Winter 1977					
18"	7,7	T,B	1,9	3.724	.086	3,6	.183	.904
30"	13,13	T,B	1,21	1.619	.217	3,18	.109	.954
42"	13,13	T,B	1,21	.005	.943	3,18	1.858	.173
90"	10,9	T,M	1,14	.047	.832	3,11	.129	.941
90"	9,9	B,M	1,13	.202	.661	3,10	.345	.794
90"	10,9	T,B	1,14	.000	.985	3,11	.309	.819

* degrees of freedom
** probability associated with F

TABLE B-2. COMPARISON OF MODELS IN TERMS OF TIME AND pH FOR TOP AND BOTTOM OF ALL PONDS (ALSO MIDDLE FOR 90" PONDS) IN SPRING 1976.

Ponds	Sample Sizes	Depth	Equality of Intercepts *d.f.	F	**P	Equality of Slopes d.f.	F	P
			Primary Effluent, Spring 1976					
18"	11,9	T,B	1,16	1.168	.296	2,14	.743	.493
90"	11,11	T,M	1,18	.010	.921	2,16	.439	.652
90"	11,11	B,M	1,18	.118	.735	2,16	1.943	.176
90"	11,11	T,B	1,18	.428	.521	2,16	1.734	.208
			Primary Effluent, Winter 1977					
18"	16,16	T,B	1,27	.075	.786	3,24	.183	.907
30"	15,15	T,B	1,25	1.694	.205	3,22	.439	.728
42"	16,16	T,B	1,27	.008	.931	3,24	.569	.641
90"	17,16	T,M	1,28	.878	.357	3,25	.557	.648
90"	17,16	B,M	1,28	.072	.789	3,25	1.414	.262
90"	17,17	T,B	1,29	.270	.607	3,26	2.707	.066

TABLE B-3a. FINAL EFFLUENT: MODELS IN TERMS OF TIME AND pH WHEN ALL DATA ARE CONSIDERED WITHIN EACH POND.

Seasons	Ponds	R^2	Standard Error	Models
Fall 75	18	.984	.478	$27.971 - 1.726p^{\alpha} - .957d^{\beta}$
	30	.975	.588	$47.144 - 3.632p - .826d$
	42	.976	.555	$22.044 - 1.248p - .916d$
	90	.959	.309	$9.712 - .001p - .489d$
Spring 76	18	.994	.306	$12.085 - {}^{*}.200p - .849d$
	30	.795	2.029	$16.982 - {}^{*}.916p - {}^{*}.255d$
	42	.982	.602	$2.662 + {}^{*}1.107p - .527d$
	90	.911	1.212	$9.069 + {}^{*}.289p - .512d$
Summer 76	18	.994	.402	$50.136 - 4.945p - 1.424d$
	30	.758	1.964	$27.611 - {}^{*}2.244p - {}^{*}.802d$
	42	.942	.724	$7.281 + {}^{*}.437p - .989d$
	90	.839	1.233	$53.792 - 5.901p - {}^{*}.029d$
Winter 76	18	.973	.511	$24.528 - 1.838p - .255d$
	30	.977	.331	$8.705 + {}^{*}.223p - .254d$
	42	.984	.218	$24.174 - 1.779p - .156d$
	90	.871	1.098	$21.034 - 1.362p - .151d$
Winter 77	18	.941	.670	$21.187 - 1.486p - .259d$
	30	.961	.388	$13.880 - .473p - .110d$
	42	.944	.512	$19.982 - 1.311p - .109d$
	90	.875	.868	$27.578 - 2.548p - .092d$

* coefficient not significant
α pH
β time (days)

TABLE B-3b. FINAL EFFLUENT: MODELS IN TERMS OF TIME AND pH WHEN DATA ARE TRUNCATED WITHIN EACH POND.

Seasons	Ponds	Truncation Points	R^2	Standard Error	Models
Fall 75	18	5.0	.903	.605	56.309 - 4.527p - .379d
	30	5.0	.924	.643	41.292 - 3.052p - .891d
	42	5.0	.966	.351	12.433 - .228p - 1.322d
	90	5.0	.945	.209	10.411 - .066p - .679d
Spring 76	18	7.0	.971	.453	36.315 - 3.368p
	30	10.0	.986	.168	16.373 - .744p - .292d
	42	10.0	.971	.139	5.088 + *.712p - .298d
	90	10.0	.945	.131	18.328 - 1.052p - .089d
Summer 76	18	2.0	.949	.225	9.583 - 1.424d
	30	4.0	.988	.482	71.895 - 7.549p - .955d
	42	6.0	.926	.518	13.216 - *.371p - .718d
	90	6.0	.943	.818	56.086 - 6.181p - *.132d
Winter 76	18	21.0	.984	.244	13.006 - *.322p - .261d
	30	21.0	.971	.300	8.922 + *.185p - .238d
	42	21.0	.969	.194	20.451 - 1.293p - .147d
	90	25.0	.802	.373	11.810 - *.178p - .087d
Winter 77	18	10.0	.810	.302	-4.191 + *1.846p - .187d
	30	25.0	.899	.346	20.395 - *1.349p - .103d
	42	25.0	.887	.375	12.326 - *.280p - .119d
	90	25.0	.478	.842	3.534 + *.642p - .094d

TABLE B-3c. FINAL EFFLUENT: MODELS IN TERMS OF TIME, pH AND TEMPERATURE WHEN WHOLE DATA ARE CONSIDERED WITHIN EACH POND.

Seasons	Ponds	R^2	Standard Error	Models
Fall 75	18	.988	.433	$29.919 - 1.745p - .991d - .985t^{\gamma}$
	30	.976	.603	$47.041 - 3.702p - .809d +^{*} .040t$
	42	.978	.558	$22.609 - 1.438p - .895d +^{*} .065t$
	90	.975	.192	$9.486 - .593p - .351d + .207t$
Winter 76	18	.983	.418	$22.876 - 1.368p - .275d - .178t$
	30	.980	.314	$10.475 +^{*} .062p - .244d -^{*} .061t$
	42	.985	.218	$24.049 - 1.714p - .157d -^{*} .038t$
	90	.827	1.140	$23.509 - 1.609p - .157d -^{*} .056t$
Winter 77	18	.973	.476	$33.757 - 2.999p - .172d - .356t$
	30	.961	.396	$13.659 -^{*} .433p - .110d -^{*} .018t$
	42	.945	.519	$20.194 - 1.363p - .112d +^{*} .043t$
	90	.881	.962	$24.410 - 2.277p - .133d +^{*} .278t$

γ temperature (C)

TABLE B-3d. FINAL EFFLUENT: MODELS IN TERMS OF TIME, pH AND TEMPERATURE WHEN DATA ARE TRUNCATED.

Seasons	Ponds	Truncation Points	R^2	Standard Error	Models
Fall 75	18	5.0	.813	.579	54.805 - *3.732p - *.675d - *.319t
	30	5.0	.936	.641	31.480 - *1.784p -1.140d - *.146t
	42	5.0	.980	.289	9.942 + *.242p -1.407d - .103t
	90	5.0	.978	.141	9.727 - .490p - .443d + .165t
Winter 76	18	21.0	.985	.250	13.668 - *.368p - .264d - *.029t
	30	21.0	.971	.309	9.345 + *.149p - .237d - *.015t
	42	21.0	.970	.199	19.328 - 1.190p - .144d - *.034t
	90	24.0	.809	.374	12.353 - *.193p - .088d - *.041t
Winter 77	18	10.0	.844	.296	.596 - *1.283p - .188d - *.140t
	30	25.0	.903	.354	19.457 - *1.193p - .103d - *.051t
	42	25.0	.893	.378	12.303 - *.236p - .119d - *.067t
	90	Excluded			

TABLE B-4a. PRIMARY EFFLUENT: MODELS IN TERMS OF TIME AND pH WHEN ALL DATA ARE USED IN EACH POND.

Seasons	Ponds	R^2	Standard Error	Models
Spring 76	30	.892	.865	7.860 + *.363p - .131d
	90	.842	.965	10.669 + *.004p - .109d
Summer 76	18	.915	.501	13.682 - *.476p - .289d
	30	.871	.734	21.116 - 1.560p - .225d
	42	.979	.285	14.615 - .614p - .265d
	90	.852	.844	13.070 - *.368p - .318d
Winter 77	18	.951	.449	14.581 - .620p - .087d
	30	.936	.590	18.581 - 1.093p - .080d
	42	.921	.458	15.241 - .636p - .067d
	90	.889	.375	11.197 - *.113p - .053d

TABLE B-4b. PRIMARY EFFLUENT: MODELS IN TERMS OF TIME AND pH WITH TRUNCATED DATA.

Seasons	Ponds	Truncation Points	R^2	Standard Error	Models
Spring 76	30	45.0	.915	.501	10.506 - *.029p - .100d
	90	45.0	.895	.366	11.017 - *.109p - .066d
Summer 76	18	8.0	.919	.395	30.728 - 2.800p - .287d
	30	15.0	.732	.773	30.284 - *2.838p - .190d
	42	15.0	.961	.284	11.581 - *.195p - .264d
	90	15.0	.803	.843	9.077 + *.225p - .366d
Winter 77	18	50.0	.938	.408	13.344 - .460p - .085d
	30	42.0	.894	.489	16.246 - .784p - .082d
	42	42.0	.901	.296	10.369 + *.002p - .063d
	90	60.0	.888	.289	8.448 + *.245p - .048d

TABLE B-4c. PRIMARY EFFLUENT: MODELS IN TERMS OF TIME, pH AND TEMPERATURE USING THE WHOLE DATA IN EACH POND.

Season	Ponds	R^2	Standard Error	Models
Winter 77	18	.954	.443	15.034 - .641p - .077d - *.078t
	30	.936	.598	18.716 - 1.092p - .075d - *.041t
	42	.934	.425	15.171 - .563p - .054d - .122t
	90	.904	.355	13.087 - *.284p - .038d - .139t

TABLE B-4d. PRIMARY EFFLUENT: MODELS IN TERMS OF TIME, pH AND TEMPERATURE WHEN THE DATA ARE TRUNCATED.

Seasons	Ponds	Truncation Points	R^2	Standard Error	Models
Winter 77	18	50.0	.941	.406	13.750 - .485p - .078d - *.058t
	30	42.0	.898	.491	16.448 - .784p - .075d - *.057t
	42	42.0	.939	.238	9.646 + *.163p - .054d - .116t
	90	60.0	.909	.266	10.829 + *.008p - .033d - .139t

TABLE B-5a. FINAL EFFLUENT: OVERALL AND PAIRWISE COMPARISON OF PONDS WITHIN EACH SEASON WITH DAYS AND pH IN THE MODEL.

Season	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Fall 75	10,10,10,12	Overall (18,30,42,90)	3,36	1.54	.22	6,30	5.34	.001
	10,10	18,30	1,16	1.71	.21	2,14	.35	.71
	10,10	18,42	1,16	2.02	.17	2,14	.78	.48
	10,12	18,90	1,18	.78	.39	2,16	8.27	.003
	10,10	30,42	1,16	1.78	.200	2,14	2.86	.09
	10,12	30,90	1,18	1.16	.29	2,16	15.61	.0002
	10,12	42,90	1,18	.28	.60	2,16	17.79	.0001
Spring 76	6,6,6,9	Overall (18,30,42,90)	3,21	5.85	.005	6,15	30.86	.000
	6,6	18,30	1,8	11.87	.009	2,6	30.26	.001
	6,6	18,42	1,8	.17	.689	2,6	38.62	.0004
	6,9	18,90	1,11	.71	.417	2,9	35.47	.0001
	6,6	30,42	1,8	.004	.95	2,6	11.01	.009
	6,9	30,90	1,11	.24	.631	2,9	12.72	.002
	6,9	42,90	1,11	6.07	.032	2,9	7.80	.011
Summer 76	4,6,8,12	Overall (18,30,42,90)	3,24	3.66	.027			
	4,6	18,30	1,6	6.37	.045			
	4,8	18,42	1,8	5.24	.051			
	4,12	18,90	1,12	13.05	.004			
	6,8	30,42	1,10	.09	.771	2,8	34.81	.0001
	6,12	30,90	1,14	9.23	.009	2,12	8.006	.006
	8,12	42,90	1,16	10.58	.005	2,14	8.86	.003

TABLE B-5a. FINAL EFFLUENT: OVERALL AND PAIRWISE COMPARISON OF PONDS WITHIN EACH SEASON WITH DAYS AND pH IN THE MODEL (CONTINUED)

Season	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Winter 76	16,18, 16,27	Overall	3,21	12.92	.000	6,65	28.64	.000
	16,18	18,30	1,30	.11	.74	2,28	4.14	.027
	16,16	18,42	1,28	24.31	.00	2,26	34.23	.000
	16,27	18,90	1,39	17.89	.000	2,37	55.26	.00
	18,16	30,42	1,30	28.89	.00	2,28	14.09	.00
	18,27	30,90	1,41	21.09	.00	2,39	25.60	.00
	16,27	42,90	1,39	2.98	.092	2,37	10.06	.000
Winter 77	10,16,18	Overall (90 excluded)	2,39	12.911	.000	4,35	1.127	.359
	10,16	18,30	1,22	20.758	.000	2,20	2.388	.118
	10,18	18,42	1,24	16.462	.001	2,22	1.203	.319
	16,18	30,42	1,30	2.173	.151	2,28	.487	.619

TABLE B-5b. FINAL EFFLUENT: COMPARISON OF MODELS WITHIN PONDS OF EACH SEASON WHEN DAYS, pH AND TEMPERATURE ARE CONSIDERED.

Season	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Fall 75	10,10,10,12	Overall (18,30,42,90)	3,35	1.554	.218	9,26	3.219	.009
	10,10	18,30	1,15	1.632	.221	3,12	.419	.743
	10,10	18,42	1,15	.562	.465	3,12	.751	.543
	10,12	18,90	1,17	2.124	.163	3,14	6.444	.006
	10,10	30 42	1,15	.256	.62	3,12	1.513	.261
	10,12	30,90	1,17	1.274	.275	3,14	7.779	.003
	10,12	42,90	1,17	1.274	.275	3,14	18.094	.000
Winter 76	16,18,16,27	Overall (18,30,42,90)	3,70	12.738	.000	9,61	18.556	.000
	16,18	18,30	1,29	.108	.745	3,26	2.718	.065
	16,16	18,42	1,27	19.255	.000	3,24	21.795	.000
	16,27	18,90	1,38	17.508	.000	3,35	36.005	.000
	18,16	30,42	1,29	27.435	.000	3,26	8.503	.000
	18,27	30,90	1,40	21.673	.000	3,37	16.398	.000
	16,27	42,90	1,38	2.387	.131	3,35	6.899	.001
Winter 77	10,16,18	Overall (18,30,42)	2,38	14.618	.000	6,32	.709	.644
	10,16	18,30	1,21	22.828	.000	3,18	1.482	.253
	10,18	18,42	1,23	18.638	.000	3,20	.834	.491
	16,18	30,42	1,29	1.975	.171	3,26	.286	.835

TABLE B-6a. FINAL EFFLUENT: COMPARISON OF SEASONS WITHIN EACH POND WHEN DAYS AND pH ARE CONSIDERED.

Pond	Sample Sizes	Seasons	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Pond 18	10,6,4,16,10	Overall (F75,SP76, SU76,W76, W77)	4,39	16.6	.000			
	10,6	F75,SP76	1,12	1.306	.275	2,10	4.211	.0471
	10,4	F75,SU76	1,10	.701	.422			
	10,16	F75,W76	1,22	9.382	.006	2,20	23.118	.000
	10,10	F75,W77	1,16	30.093	.000	2,14	7.046	.0076
	6,4	SP76,SU76	1,6	37.819	.001			
	6,16	SP76,W76	1,18	.401	.535	2,16	25.977	.000
	6,10	SP76,W77	1,12	7.045	.021	2,10	12.287	.002
	4,16	SU76,W76	1,16	14.309	.002			
	4,10	SU76,W77	1,10	2.895	.119			
	16,10	W76,W77	1,22	8.341	.008	2,20	10.732	.001
Pond 30	10,6,6,18,16	Overall (F75,SP76, SU76,W76, W77)	4,49	7.607	.0001	8,41	45.244	.000
	10,6	F75,SP76	1,12	2.517	.139	2,10	17.328	.001
	10,6	F75,SU76	1,12	5.490	.037	2,10	8.547	.007
	10,18	F75,W76	1,24	1.607	.217	2,22	56.221	.000
	10,16	F75,W77	1,22	53.617	.000	2,20	8.986	.002
	6,6	SP76,SU76	1,8	1.814	.215	2,6	110.347	.000
	6,18	SP76,W76	1,20	.717	.407	2,18	7.881	.004
	6,16	SP76,W77	1,18	3.747	.069	2,16	1.926	.178
	6,18	SU76,W76	1,20	1.301	.268	2,18	189.91	.000
	6,16	SU76,W77	1,18	18.655	.000	2,16	40.902	.000
	18,16	W76,W77	1,30	8.169	.008	2,28	19.129	.000

TAPLE B-6a. FINAL EFFLUENT: COMPARISON OF SEASONS WITHIN EACH POND WHEN DAYS AND pH ARE CONSIDERED (CONTINUED).

Pond	Sample Sizes	Seasons	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Pond 42	10,6,8,16,18	Overall	4,51	10.125	.000	8,43	18.298	.000
	10,6	F75,SP76	1,12	1.748	.211	2,10	46.399	.000
	10,8	F75,SU76	1,14	.863	.369	2,12	6.538	.012
	10,16	F75,W76	1,22	14.041	.0011	2,20	53.913	.000
	10,18	F75,W77	1,24	14.429	.001	2,22	30.306	.000
	6,8	SP76,SU76	1,10	6.91	.025	2,8	12.722	.003
	6,16	SP76,W76	1,18	10.177	.005	2,16	4.982	.021
	6,18	SP76,W77	1,20	.041	.841	2,18	1.368	.279
	8,16	SU76,W76	1,20	7.633	.012	2,18	29.261	.000
	8,18	SU76,W77	1,22	.507	.484	2,20	21·223	.000
	16,18	W76,W77	1,30	4.647	.039	2,28	3.139	.059
Pond 90	12,9,12,27	Overall (F75,SP76, SU76,W76)	3,54	13.568	.000	4,48	55.811	.000
	12,9	F75,SP76	1,17	39.61	.000	2,15	47.576	.000
	12,12	F75,SU76	1,20	.003	.961	2,18	30.569	.000
	12,27	F75,W76	1,35	50.731	.000	2,33	23.023	.000
	9,12	SP76,SU76	1,17	10.357	.005	2,15	11.815	.001
	9,27	SP76,W76	1,32	1.32	.259	2,30	1.175	.323
	12,27	SU76,W76	1,35	15.906	.000	2,33	48.278	.000

TABLE B-6b. FINAL EFFLUENT: COMPARISON OF SEASONS WITHIN EACH POND WHEN DAYS, pH AND TEMPERATURE ARE CONSIDERED.

Pond	Sample Sizes	Seasons	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Pond 18	10,16,10	Overall (F75,W76,W77)	2,30	12.314	.000	6,24	11.764	.000
	10,16	F75,W76	1,21	18.152	.000	3,18	16.038	.000
	10,10	F75,W77	1,15	48.343	.000	3,12	4.812	.020
	16,10	W76,W77	1,21	2.357	.139	3,18	7.448	.002
Pond 30	10,18,16	Overall (F75,W76,W77)	2,38	10.338	.000	6,32	19.642	.000
	10,18	F75,W76	1,23	5.483	.028	3,20	31.197	.000
	10,16	F75,W77	1,21	45.651	.000	3,18	6.789	.003
	18,16	W76,W77	1,29	1.148	.293	3,26	12.058	.000
Pond 42	10,16,18	Overall (F75,W76,W77)	2,38	6.714	.003	6,32	17.758	.000
	10,16	F75,W76	1,21	12.466	.002	3,18	46.491	.000
	10,18	F75,W77	1,23	11.230	.003	3,20	22.991	.000
	16,18	W76,W77	1,29	.395	.535	3,26	2.398	.091
Pond 90	12,27	F75,W76	1,34	24.753	.000	3,31	13.059	.000

TABLE B-7a. PRIMARY EFFLUENT: COMPARISON OF PONDS WITHIN EACH SEASON WHEN DAYS AND pH ARE CONSIDERED.

Seasons	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Spring 76	18,27	30,90	1,41	10.31	.003	2,39	7.39	.002
Summer 76	10,14,13,21	Overall (18,30,42,90)	3,52	.28	.84	6,46	1.87	.11
	10,14	18,30	1,20	1.90	.18	2,18	.48	.63
	10,13	18,42	1,19	.01	.91	2,17	6.01	.01
	10,21	18,90	1,27	1.40	.25	2,25	.85	.44
	14,13	30,42	1,23	.79	.39	2,21	.86	.44
	14,21	30,90	1,31	.22	.64	2,29	2.56	.09
	13,21	42,90	1,30	.001	.98	2,28	1.29	.289
Winter 77	30,26,28,48	Overall (18,30,42,90)	3,126	29.35	.000	6,120	12.63	.000
	30,26	18,30	1,52	13.07	.001	2,50	.81	.45
	30,28	18,42	1,54	74.82	.000	2,52	11.78	.000
	30,48	18,90	1,74	71.59	.000	2,72	26.37	.000
	26,28	30,42	1,50	10.64	.002	2,48	10.58	.000
	26,48	30,90	1,70	4.85	.031	2,68	16.86	.000
	28,48	42,90	1,72	2.97	.09	2,70	5.34	.007

TABLE B-7b. PRIMARY EFFLUENT: COMPARISON OF PONDS WITHIN EACH SEASON WHEN DAYS, pH AND TEMPERATURE ARE CONSIDERED.

Seasons	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Winter 77	30,26,28,48	Overall (18,30,42,90)	3,125	32.945	.000	9,116	8.887	.000
	30,26	18,30	1,51	12.645	.001	3,48	.469	.705
	30,28	18,42	1,53	77.511	.000	3,50	9.518	.000
	30,48	18,90	1,73	84.101	.000	3,70	16.708	.000
	26,28	30,42	1,49	11.903	.001	3,46	8.343	.000
	26,48	30,90	1,69	8.131	.006	3,66	10.637	.000
	28,48	42,90	1,71	8.748	.004	3,68	4.532	.006

TABLE B-8. PRIMARY EFFLUENT: COMPARISON OF SEASONS WITHIN EACH POND WHEN DAYS AND pH ARE CONSIDERED.

Pond	Sample Sizes	Seasons	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Pond 18		SUM76,W77	1,38	5.99	.019	2,36	16.81	.000
Pond 30		Overall SP76,SUM76,W77	2,55	18.12	.000	4,51	4.48	.004
		SP76,SUM76	1,30	15.10	.001	2,28	3.89	.032
		SP76,W77	1,42	2.66	.11	2,40	3.49	.04
		SUM76,W77	1,36	38.32	.000	2,34	8.97	.001
Pond 42		SUM76,W77	1,37	40.97	.000	2,35	62.24	.000
Pond 90		Overall SP76,SUM76,W77	2,91	25.80	.000	4,87	39.99	.000
		SP76,SUM76	1,44	19.79	.000	2,42	39.99	.000
		SP76,W77	1,71	20.92	.000	2,69	8.77	.000
		SUM76,W77	1,65	35.38	.000	2,63	66.97	.000

TABLE B-9a. PONDWISE COMPARISON OF FINAL AND PRIMARY EFFLUENTS, MODELS WITHOUT TEMPERATURE.

Seasons	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Spring 76	6,18	F30",P30"	1,20	.549	.467	2,18	5.836	.011
	9,27	F90",P90"	1,32	.154	.697	2,30	2.464	.102
Summer 76	4,10	F18",P18"	1,10	.113	.743			
	6,14	F30",P30"	1,16	18.872	.001	2,14	9.379	.003
	8,13	F42",P42"	1,17	.0001	.993	2,15	16.585	.000
	12,21	F90",P90"	1,29	.423	.521	2,27	28.172	.000
Winter 77	10,30	F18",P18"	1,36	7.189	.011	3,34	2.177	.129
	16,26	F30",P30"	1,38	5.394	.026	2,36	1.326	.278
	18,28	F42",P42"	1,42	38.128	.000	2,40	13.663	.000

Note: The F and P preceding the pond depths refers to the final and primary effluents.

TABLE B-9b. PONDWISE COMPARISON OF FINAL AND PRIMARY EFFLUENT, MODELS WITH TEMPERATURE.

Seasons	Sample Sizes	Ponds	Equality of Intercepts d.f.	F	P	Equality of Slopes d.f.	F	P
Winter 77	10,30	F18",P18"	1,35	7.912	.008	3,32	1.781	.171
	16,26	F30",P30"	1,37	5.578	.024	3,34	1.128	.352
	18,28	F42",P42"	1,41	39.816	.000	3,38	13.728	.000

APPENDIX C

POND PHYSICAL AND CHEMICAL DATA

ABBREVIATIONS FOR APPENDIX C

B	bottom
COD	chemical oxygen demand
mg/l	milligrams per liter
T	top
TKN	total kjeldahl nitrogen
TOC	total organic carbon
TSS/VSS	total suspended solids/volatile suspended solids

TABLE C-1. FALL, 1975 - 18" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	80/58	70/64	29	33	102	112	2.7	2.1	.19	.18	1.80	1.83
1 day	24/22	26/26	31	25								
2 days	24/29	33/29	18	19								
3 days	40/36	32/33	17	19								
6 days	35/28	38/32	22	21	80	127	.65	.32	<.05	<.05	1.12	.88

TABLE C-2. FALL, 1975 - 30" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	78/58	88/66	20	17	104	102	5.7	5.9	.16	.15	1.74	1.81
1 day	55/48	58/52	27	23								
2 days	58/53	63/52	31	33								
3 days	73/49	55/37	27	25								
6 days	66/49	54/50	18	18	71	78	.65	.65	.20	.23	1.13	1.22

TABLE C-3. FALL, 1975 - 42" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	91/64	90/65	32	30	111	109	6.5	6.5	.37	.30	2.12	2.00
1 day	64/52	64/51	25	20								
2 days	62/52	65/54	29	20								
3 days	69/51	72/55	23	19								
5 days	63/49	67/49	19	20	83	80	1.84	1.30	2.1	2.0	2.46	2.43

TABLE C-4. FALL, 1975 - 90" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	20/10	20/19	12	13	37	36	8.8	13.2	.05	.07	3.65	3.65
1 day	13/6	10/8	9	10								
2 days	5/8	7/5	7	10								
3 days	14/6	11/5	9	9								
5 days	7/5	9/5	9	11	41	36	9.9	10.5	.04	.04	5.3	5.3
7 days	14/11	11/7	19	12								

TABLE C-5. WINTER, 1976 - 18" Final Effluent Pond (Series 1)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	157.5/27.6	195.6/35.4	25	27								
4 hours	47.8/11.4	82.8/17.0			58	63	13.8	15.3	.07	.07	4.7	4.7
1 day	24.5/9.3	40.2/10.6	27	21								
6 days	8.6/4.2	9.1/4.0	23	21	54	53	11.2	10.6	.17	.17	5.3	5.0
13 days	1.2/1.6	1.2/2.0	18	17			12.0	11.8	.14	.15	3.5	3.2

TABLE C-6. WINTER, 1976 - 30" FINAL EFFLUENT POND (SERIES 1)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	545.4/77.8	607.6/84.0	29	31								
4 hours	145.3/21.8	299.9/40.4			51	54	15.3	12.8	.14	.15	4.7	3.2
1 day	58.5/13.9	117.0/21.3	28	31								
6 days	16.7/15.1	111.4/15.8	21	21	57	48	10.3	10.4	.21	.21	4.1	4.1
13 days		31.2/10.0	17	21			10.4	10.8	.29	.27	3.8	3.8

TABLE C-7. WINTER, 1976 - 42" FINAL EFFLUENT POND (SERIES 1)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	16.0/9.1	21.2/13.4	28	26								
4 hours	10.2/8.0	16.3/11.8			69	53	12.7	11.5	.06	.05	2.9	2.7
1 day	7.0/5.8	13.6/7.6	22	26								
6 days	17.4/5.2	10.7/5.1	23	23	47	50	10.3	10.9	.16	.16	3.2	2.9
13 days	2.2/2.4	1.6/2.0	23	19			12.0	12.0	.10	.10	3.8	3.8

TABLE C-8. WINTER, 1976 - 90" FINAL EFFLUENT POND (SERIES 1)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	39.8/15.6	38.4/25.5	27	30								
4 hours	9.3/7.4	28.9/13.7			53	78	12.8	12.9	.06	.07	2.8	3.5
1 day	7.4/5.5	14.1/8.0	23	27								
6 days	6.8/4.1	17.1/4.9	20	22	64	54	11.8	11.3	.17	.17	2.9	2.9
13 days	1.6/2.0	2.8/3.8	19	22			11.4	11.3	.06	.06	4.1	3.8

TABLE C-9. WINTER, 1976 - 18" FINAL EFFLUENT POND (SERIES 3)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	31.8/10.8	77.6/17.6	27	25								
4 hours	10.5/7.3	28.0/15.4			60	88	13.7	12.2	.07	.08	4.1	4.7
1 day	10.6/6.3	18.0/9.7	25	45								
6 days	8.3/4.0	7.5/3.5	23	19	38	52	18.2	13.6	.17	.16	4.7	4.4
13 days	3.2/2.8	2.2/2.0	18	20	47	46	10.6	10.6	.14	.14	3.8	4.0
19 days		12.3/4.2	18	18	34	35	13.1	13.1	.12	.14		
24 days	19.2/17.0	25.9/18.4	21	22								
31 days	135.1/123.3	18.1/10.2			269	57					1.9	1.9

TABLE C-10. WINTER, 1976 - 30" FINAL EFFLUENT POND (SERIES 3)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	363.5/28.8	196.0/29.8	34	26								
4 hours	50.0/12.2	109.5/17.9			67	77	13.7	11.2	.29	.31	2.5	2.2
1 day	35.3/10.3	53.7/15.0	24	23								
6 days	16.2/6.3	30.3/4.7	19	19	43	55	11.5	10.8	.40	.44	4.1	4.4
13 days	6.0/4.6	6.0/5.2	17	18	41	43	10.6	10.6	.15	.15	3.4	3.2
19 days	33.5/25.1	40.9/30.9	29	27	68	66	12.5	11.8	.16	.18		
24 days	53.9/44.0	51.0/40.5	30	31								
31 days	68.8/61.3	24.1/17.6			194	86					2.2	2.5

TABLE C-11. WINTER, 1976 - 42" FINAL EFFLUENT POND (SERIES 3)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	17.3/11.4	26.7/10.1	29	31								
4 hours	4.8/4.8	13.4/13.4			55	85	12.1	12.2	.06	.05	4.7	5.0
1 day	8.4/6.4	10.7/14.7	27	23								
6 days	9.6/5.7	6.0/4.1	20	21	48	54	19	10.2	.13	.14	3.6	3.6
13 days	2.8/2.8	3.2/2.6	18	18	40	40	10.9	10.9	.13	.13	2.9	2.8
19 days		15.0/4.4	21	21	72	71	16.8	16.3	.11	.10		
24 days	12.0/8.7	15.9/9.1	19	19								
31 days	85.6/73.6	23.8/17.3	32	37	146	74					2.3	2.3
45 days	55.7/50.4	56.3/45.0	45	45	181	183					2.1	2.2

TABLE C-12. WINTER, 1976 - 90" FINAL EFFLUENT POND (SERIES 3)

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	87.5/20.7	103.6/15.5	25	26								
4 hours	5.8/5.8	57.0/23.3			64	64	10.2	8.3	.09	.08	3.8	3.8
1 day	6.4/5.2	16.2/8.2	28	27								
6 days	11.7/6.1	7.9/8.4	22	20	65	50	11.8	11.5	.16	.16	3.8	4.7
13 days	5.8/4.0	5.4/4.2	16	19	39	39	10.6	10.8	.11	.10	2.7	2.8
19 days		5.9/3.6	19	16	33	37	19.3	18.1	.09	.09		
24 days	6.0/3.1	4.2/2.8	21	17								
31 days	109.0/94.6	5.7/4.5	34	26	210	47					2.3	
45 days	74.6/68.4	14.7/12.5	57	26	216	115					2.6	

TABLE C-13. SPRING, 1976 - 18" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	65.6/26.5	81.0/29.3	27	30	59	59	17.0	17.6	.036	.1	6.3	6.3
3 days	162.2/9.7	20.0/13.2										
6 days	47.2/35.8	62.5/46.7	43	41	122	122	4.9	5.6	.11	.12	4.0	4.2
15 days	63.0/53.2	62.6/48.8	52	56	142	137	2.8	2.8	.130	.125		1.2

TABLE C-14. SPRING, 1976 - 30" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	107.0/39.0	153.8/33.2	34	30	73	78	14.8	15.9	.11	.160	6.7	6.7
3 days	30.2/17.5	41.9/17.5										
6 days	57.5/40.9	20.9/15.0	39	39	120	81	7.7	11.2	.12	.1	5.0	7.0
15 days	73.4/53.0	68.4/48.2	56	51	138	56	3.4	5.5	.295	.140	1.5	4.2
22 days	80.0/63.6	75.0/61.0	49	49	98	100	2.3	2.7	.094	.094	1.7	2.0
35 days	79.0/60.0		54		121							1.0
REFILL												
1 hour	86.6/51.4	279.0/62.4	49	42	105	148					3.0	4.7
3 days	75.0/59.0	65.0/46.0										
3 days	66.6/48.6	71.4/52.0	51	49	97	95			.1	.137	1.0	2.0
15 days	69.4/59.8	92.4/45.0	50	58	148	164			.085	.093	0.9	1.2

TABLE C-15. SPRING, 1976 - 42" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	19.3/11.3	21.0/11.4	30	29	58	64	17.6	13.2	.1	.1	6.0	5.8
3 days	11.0/4.4	7.1/4.5										
6 days												
15 days	99.4/32.0	59.2/45.2	53	43	133	102	4.3	12.0	.240	.145	1.8	6.5
22 days	61.0/49.6	67.2/50.4	39	42	72	76	2.8	3.2	.13	.13	2.0	2.0

TABLE C-16. SPRING, 1976 - 90" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	19.3/10.2	20.0/10.2	21	27	62	65	18.1	18.1	.16	.16	6.0	6.1
3 days	5.4/2.4	13.3/7.9										
6 days												
15 days	50.2/39.6	15.5/10.8	63	32	151	67	5.7	13.5	.20	.280	2.3	5.5
22 days	51.4/48.6	58.0/43.0	39	35	62	62		7.1	.7	.72	3.7	3.8
35 days												
REFILL												
1 hour	85.0/55.4	83.4/60.4	43	44	91	100					4.7	4.7
3 days	54.0/40.2	50.8/49.8										
8 days	31.0/19.4	44.0/40.0	51	34	33	50			8.6	10.8	1.7	3.2
15 days	27.4/14.0	41.4/35.8	26	28	47	50			4.15	2.88	1.2	3.2

TABLE C-17. SPRING, 1976 - 30" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate Ortho/Total (mg/l) T	Phosphate Ortho/Total (mg/l) B
1 hour	128.0/59.8	137.0/57.5	100	95	321	206	25.3	25.3	.083	.083	10.2/10.4	10.0/9.0
3 days	54.6/39.8	53.6/37.3	75	85	223	232	31.2	30.4	.086	.081	10.0/9.0	10.2/9.2
6 days	37.5/26.2	45.0/27.8	75	70	175	198	22.5	23.2	.086	.070	9.2/9.0	9.8/9.0
15 days	46.0/36.4	28.7/24.2	100	35	173	140	8.5	21.9	.1	.12	6.8/6.8	9.7/9.3
22 days	54.4/42.0	52.0/43.0	46	47	105	109	7.1	6.9	.047	.047	7.0/7.8	7.0/7.4
29 days	25.4/22.6	30.6/21.0	35	40	135	133	6.5	6.5	.073	.082	7.2/7.5	7.1/7.3
34 days	119.2/112.4	16.5/12.9	86	43	286	94			.093	.07	7.5/8.5	7.5/7.1
43 days	27.6/25.4	25.0/20.4	48	48	96	95				.079	5.5/5.7	6.8/7.3
50 days	151.6/39.6	33.4/32.8	82	35	263	113			.15	.08	4.7/6.3	6.7/6.8

TABLE C-18. SPRING, 1976 - 90" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	Bottom	TOC (mg/l) T	B	COD (mg/l) T	B	TKN (mg/l) T	B	NO_2/NO_3 (mg/l) T	B	Phosphate Ortho/Total (mg/l) T	B
1 hour	138.0/86.4	165.4/109.0	110	95	242	296	28.0	31.3	.088	.084	9.3/10.4	9.7/9.6
3 days	47.0/38.0	46.2/38.5	95	95	209	216	35.7	34.2	.086	.097	8.8/8.4	8.6/8.4
6 days	32.6/24.4	38.2/24.4	85	90	198	240	26.0	30.2	.076	.11	9.2/8.4	8.7/9.0
15 days	37.3/31.4	30.2/25.2	80	85	164	198	22.7	28.4	.10	.160	8.1/7.4	9.0/8.0
22 days	77.2/60.0	63.6/48.0	59	47	151	125	30.4	29.2	.076	.7	8.5/9.6	8.2/9.3
29 days	54.4/44.0	61.8/27.2	43	72	149	252	18.5	31.5	.073	.15	5.8/4.3	10.1/9.5
34 days	104.4/95.1	145.0/134.5	76	49	208	131			.15	.133	7.5/8.5	7.5/8.5
43 days	70.8/56.8	47.6/40.0	31	58	113	128			.1	.175	6.0/6.8	8.5/9.6
50 days	41.6/28.0	81.0/60.4	31	62	100	203			.089	.22	6.7/6.8	10.2/8.4

TABLE C-19. SUMMER, 1976 - 18" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	Bottom	TOC (mg/l) T	B	COD (mg/l) T	B	TKN (mg/l) T	B	NO_2/NO_3 (mg/l) T	B	Total Phosphate (mg/l) T	B
1 hour	17.0/13.0	30.1/16.2	26	30	71	69	8	12	.105	.12	3.6	5.3
3 days	71.8/47.8	44.0/27.6	33	29	96	77	2	2			3.6	3.3
7 days	11.0/8.0	8.0/8.6	16	18	60	53			.03	.03	2.8	2.5

TABLE C-20. SUMMER, 1976 - 30" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	12.0/8.5	19.0/11.6	24	27	66	51	15	13	.12	.125	5.5	5.5
3 days	32.2/25.8	28.8/19.4	25	23	65	63	5	6			4.0	5.2
7 days	44.0/22.0	48.0/27.0	30	32	83	87			.145	.14	2.5	3.8

TABLE C-21. SUMMER, 1976 - 42" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	8.3/5.2	13.4/8.1	49	25	95	54	12	16	.12	.13	5.8	5.5
3 days	16.9/11.0	82.5/65.5	20	13	53	48	10	12			4.8	4.7
7 days	5.0/5.0	4.0/3.0	30	13	47	44			.22	.21	3.7	3.7

TABLE C-22. SUMMER, 1976 - 90" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	10.8/7.2	18.4/9.6	26	25	45	47	16	14	.12	.12	5.3	5.1
3 days	18.7/15.5	8.0/7.0	16	19	63	52	8	8			4.7	4.4
7 days	13.5/11.0	15.0/11.0	22	18	51	60	8	8	.13	.135	3.5	4.3
10 days		17.0/16.5		52		86		14		.07		4.9
13 days		23.0/23.0		38		123						5.8
REFILL												
1 hour	25.0/19.0	48.0/33.0	20	28	41	46					5.2	5.2
5 days	21.0/18.0	19.0/15.0	22	22	33	38	3.9	8.2	.68	.03	4.8	5.8
10 days	16.0/15.0	26.0/21.0	28	26	65	70	2.2	5.3	2.6	.091	3.3	5.7

TABLE C-23. SUMMER, 1976 - 18" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	77.0/62.7	94.0/66.4	85	83	224	226	20	21	.13	.15	7.5	7.8
3 days	40.3/37.1	167.0/106.0	60	80	163	264					6.8	8.2
7 days	68.0/61.0	68.0/54.0	50	53	175	177	6	5			5.8	6.2
10 days	81.0/74.0	144.0/102.0	61	75	216	265	5	5	.12	.1	7.4	7.2
13 days	112.0/104.0	124.0/110.0	73	77	258	256	4	2.5	.12	.125	6.3	10.5

TABLE C-24. SUMMER, 1976 - 30" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Total Phosphate (mg/l) T	Total Phosphate (mg/l) B
1 hour	71.5/70.5	154.2/96.0	70	85	200	294	18	21	.1	.12	6.3	8.5
3 days	147.0/126.0	43.4/34.6	85	55	302	122					9.5	7.8
7 days	64.0/56.0	114.0/48.0	53	45	154	164	10	11	.07	.07	7.6	7.8
10 days	65.0/59.0	62.0/56.0	44	48	183	156	10	11	.10	.10	7.8	8.1
13 days	31.0/30.0	37.0/33.0	42	37	140	159	7.5	7.5			8.2	8.0

TABLE C-25. SUMMER, 1976 - 42" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) BT	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	73.6/54.6	97.4/70.4	67	91	199	252	21.5	22.5	.07	.13	7.7	8.1
3 days	45.9/40.2	38.5/36.1	65	78	170	238					8.2	9.2
7 days	70.0/58.0	72.0/60.0	45	65	167	206	14	19	.07	.13	7.3	8.2
10 days	60.0/54.0	59.0/55.0	52	52	133	195	12	16	.09	.13	6.5	9.0
13 days	144.0/122.0	54.0/50.0	78	43	347	149	10	9			9.2	7.0

TABLE C-26. SUMMER, 1976 - 90" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Phosphate (mg/l) T	Phosphate (mg/l) B
1 hour	63.5/51.4	443.0/254.0	70	184	201	417	26	22	.12	.105	7.2	10.5
3 days	35.1/32.0	62.8/46.7	55	150	165	470					7.0	11.3
7 days	36.0/32.0	28.0/25.0	42	128	139	402	23	60	.1	.75	6.6	11.0
10 days	23.0/21.0	28.0/25.0	33	99	119	317	18	42	.10	.22	6.2	8.8
13 days	33.0/33.0	43.0/37.0	38	82	154	258	14	28			9.2	6.0
REFILL												
1 hour	94.0/72.0	108.0/78.0	58	56	103	105					6.7	6.7
5 days	45.0/40.0	51.0/42.0	48	50	85	75	11.0	19.5	.06	.06	7.0	8.0
10 days	40.0/38.0	77.0/56.0	38	36	40	136	10.2	10.6	.04	.05	5.7	6.0
14 days	56.0/50.0	48/40	32	38	116	133	11.5	12.0	.02	.02	6.1	6.0
23 days	34/30	35/31	39	38	110	119	8.0	8.0	.07	.16	5.4	5.8

TABLE C-27. WINTER, 1977 - 18" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	58/43	69/32	53	40	122	88	16	15	.040/.25	.045/.205	3.5	3.5
1 day							17	15	.045/.215	.045/.215		
3 days	21/11	17/11	26	29								
6 days	13/10	9/9	16	27	61	93	14	14	.035/.195	.035/.465	4.2	4.0
13 days					52	44	15	14	.030/.210	.030/.19	4.4	4.4
20 days	25/18	21/7	33	35	56	68	9	9	.025/.215	.025/.205	5.4	4.9
23 days							3.2	4.1	.045/.235	.025/.205		
27 days	15/13	17/13	53	61	139	171	2.4	2.3	.020/.090	.025/.085	3.0	4.3
34 days	51/45	68/57	37	38	160	156					2.0	1.5
41 days	35/29	62/47	41	47	299	144	1.8	3.6	.010/.070	.015/.065	1.5	3.7

TABLE C-28. WINTER, 1977 - 30" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	85/39	157/85	40	55	123	144	19	13	.045/.235	.030/.130	3.7	4.4
1 day							19	13	.045/.225	.055/.225		
3 days	19/12	34/20	38	38								
6 days	19/12	16/12	23	26	105	105	15	19	.010/.100	.010/.040	4.6	4.6
13 days		12/9			56	56	17	17	.010/.090	.010/.080	5.8	5.5
20 days	27/20	31/19	37	32	56	56	17	17	.010/.060	.010/.060	7.2	7.4
23 days							14	14	.010/.065	.010/.060		
27 days	20/16	41/25	31	28	60	72					5.5	6.0
34 days	81/69	43/35	36	38	144	84	8.4	8.0	.010/.100	.010/.070	4.5	4.5
41 days	68/58	46/36	39	32	199	129	5.1	3.0	.010/.070	.010/.080	4.0	3.7

TABLE C-29. WINTER, 1977 - 42" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	145/90	170/100	68	70	213	208	17	19	.050/.215	.050/.205	4.9	5.5
1 day							19	18	.060/.245	.060/.255		
3 days	28/19	56/34	31	44								
6 days	21/16	24/18	29	38	117	80	16	17	.010/.050	.010/.040	4.5	4.8
13 days	18/8	25/14			60	88	18	17	.010/.070	.010/.070	5.1	5.8
20 days	28/22	30/26	34	32	48	48	17	17	.010/.055	.010/.065	6.6	6.6
23 days							17	16	.010/.055	.010/.060		
27 days	27/29	34/27	27	27	60	60					4.8	4.8
34 days	17/15	18/16	26	20	56	56	14	13	.010/.070	.010/.070	4.3	4.3
41 days	62/54	94/30	37	34	25	21	9.4	8.4	.020/.080	.015/.065	4.3	4.0

TABLE C-30. WINTER, 1977 - 90" FINAL EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	75/45	71/40	40	40	137	121	17	18	.045/.195	.045/.195	4.7	4.8
1 day							18	17	.055/.225	.055/.225		
3 days	9/6	23/15	33	38								
6 days	14/10	71/69	29	29	64	60	18	17	.060/.135	.060/.220	4.7	4.6
13 days	10/9	10/5			52	60	16	15	.015/.105	.015/.135	5.1	5.7
20 days	134/26	135/43	30	23	40	40	17	18	.010/.055	.010/.060	5.7	5.7
23 days							16	16	.010/.065	.025/.050		
27 days	54/47	32/24	29	29	72	68					4.3	4.0
34 days	30/27	47/37	21	24	68	76	13	13	.015/.085	.010/.080	4.2	4.0
41 days	48/48	40/34	32	34	22	22	12	12	.025/.105	.020/.110	4.3	4.5

TABLE C-31. WINTER, 1977 - 18" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	138/59	200/77	50	51	240	248	20	19	.015/.105	.015/.075	6.5	7.0
1 day							18	21	.01/.07	.01/.08		
3 days	76/55	74/56	65	76								
6 days	50/30	50/42	66	78	190	190	18	19	.01/.09	.01/.09	8.4	8.7
13 days	40/34	48/38	60	62	144	148	20	20	.015/.06	.015/.095	8.9	9.2
20 days	15/14	19/18	58	54	128	119	19	20	.010/.120	.010/.130	10.6	11.2
23 days							15	17	.015/.065	.015/.105		
27 days	97/58	109/76	52	54	111	111					9.7	8.7
34 days	10/9	9/9	20	20	76	80	14	16	.015/.065	.015/.065	7.5	7.5
41 days	71/52	60/40	39	38	124	128	11	10	.020/.070	.015/.085	7.6	7.2
48 days	108/104	80/192	35	29	211	160	3.3	3.0	.050/.055	.020/.100		
56 days	30/28	33/28	40	32			3.5	3.2	.025/.055	.020/.040	1.6	1.4

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THE SURVIVAL OF HUMAN ENTERIC VIRUSES IN HOLDING PONDS.(U)
JAN 78 B P SAGIK, S W FUNDERBURG, B E MOORE DAMD17-75-C-5062

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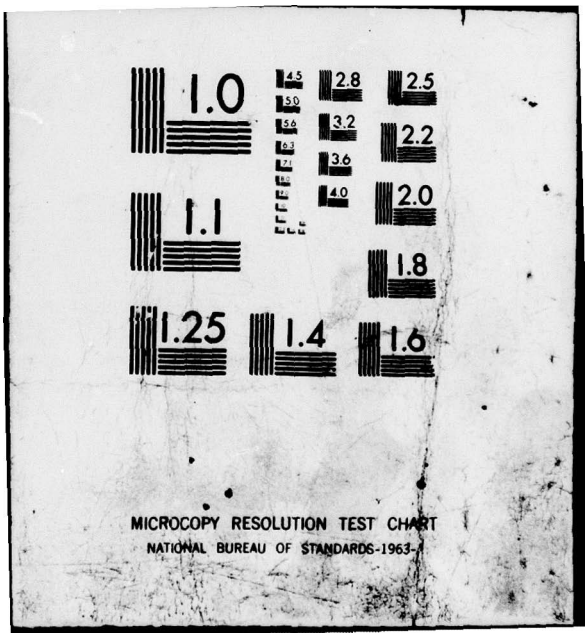
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TABLE C-32. WINTER, 1977 - 30" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	137/83	169/87	66	75	243	283	20	25	.015/.095	.010/.070	7.1	7.3
1 day							24	23	.010/.07	.010/.070		
3 days	54/48	84/66	66	66								
6 days	48/42	74/58	74	70	190	201	19	19	.010/.110	.010/.090	7.9	8.6
13 days	54/40	58/48	62	80	156	168	21	21	.015/.085	.015/.080	9.2	9.5
20 days	15/6	13/7	44	52	116	124	20	19	.015/.085	.015/.095	11.2	10.9
23 days							18	18	.015/.085	.015/.095		
27 days	32/18	24/19	46	38	92	115					7.8	7.8
34 days	22/16	92/49	20	31	84	136	12	13	.015/.055	.020/.060	7.2	7.5
41 days	33/19	60/42	34	41	87	137	6.8	8.6	.010/.040	.015/.055	5.7	6.0
48 days	25/17	56/50	33	40	56	108	3.3	1.5	.015/.075	.020/.070		
56 days	23/17	48/40	30	34			2.7	3.7	.010/.050	.030/.030	1.9	3.0

TABLE C-33. WINTER, 1977 - 42" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	135/85	164/102	96	100	356	362	24	23	.010/.120	.015/.105	8.1	8.7
1 day							23	23	.010/.080	.010/.090		
3 days	64/60	82/74	67	84								
6 days	68/54	68/54	94	90	206	230	20	21	.010/.090	.010/.090	8.8	9.5
13 days	48/42	56/46	80	94	168	224	20	26	.010/.110	.015/.125	9.1	10.6
20 days	13/7	11/6	60	64	164	180	22	23	.015/.115	.015/.115	12.0	11.2
23 days							20	22	.020/.140	.020/.120		
27 days	7/6	19/17	54	64	147	159					8.1	8.1
34 days	31/22	33/25	33	31	124	128	19	19	.020/.140	.025/.145	7.5	7.3
41 days	32/35	92/76	39	35	108	258	14	13	.015/.085	.025/.075	6.8	8.1
48 days	142/128	38/33	33	38	76	244	7.2	7.8	.010/.190	.045/.035		
56 days	52/42	35/28	29	26			11	10	.080/.120	.040/.080	4.9	8.1
63 days	148/64	148/64	32	38	202	262					3.7	8.9
72 days	32/32	54/54	32	34	136	168					8.9	8.3

TABLE C-34. WINTER, 1977 - 90" PRIMARY EFFLUENT POND

Time	TSS/VSS (mg/l) Top	TSS/VSS (mg/l) Bottom	TOC (mg/l) T	TOC (mg/l) B	COD (mg/l) T	COD (mg/l) B	TKN (mg/l) T	TKN (mg/l) B	NO_2/NO_3 (mg/l) T	NO_2/NO_3 (mg/l) B	Ortho Phosphate (mg/l) T	Ortho Phosphate (mg/l) B
1 hour	200/160	195/145	110	106	410	390	26	27	.010/.090	.010/.090	8.1	8.1
1 day							28	24	.010/.090	.010/.080		
3 days	86/68	82/60	86	86								
6 days	60/54	64/54	94	90	226	230	24	23	.010/.090	.015/.085	8.6	9.4
13 days			82	84	210	240	26	26	.015/.115	.015/.125	9.4	9.9
20 days	7/6	8/7	56	72	196	231	28	30	.015/.135	.015/.135	10.9	11.4
23 days							28	28	.040/.100	.015/.185		
27 days	16/14	12/8	84	82	226	198					8.7	8.1
34 days	47/44	31/28	52	50	208	176	29	27	.035/.155	.125/.145	8.4	7.8
41 days	62/51	46/36	36	33	204	175	31	26	.020/.110	.025/.105	9.2	8.3
48 days	86/21	50/43	34	31	189	145	25	27	.020/.100	.020/.100		
56 days	53/41	50/48	19	18			26	25	.030/.110	.030/.090	9.2	9.2
63 days	151/24	151/100	20	28	147	222					8.9	10.0
72 days	82/82	62/62	24	21	148	119					10.6	10.0

REFERENCES CITED

American Society of Civil Engineers, 1959. Sewage Treatment Plant Design, Manuel of Engineering Practice No. 36. American Society of Civil Engineers (New York).

Annett, C.S., Stephenson, M.E. & F.M. D'dtri, 1974. Phosphorus and nitrate budgets in a waste stabilization system. In: Energy, Ecology and Society, Symp., Proc., Michigan State Univ., East Lansing p. 117-123.

APHA, Standard Methods for the Examination of Waste and Wastewater, 14th edition, Washington, 1976.

Assenzo, J.R. and Reid, G.W., 1966, Removing nitrogen and phosphorus by biooxidation ponds in Central Oklahoma. Water and Sewage Works 113(8):294-298.

Barnes, G.E., 1963. Oxidation Ponds for the treatment of sewage and wastes. In: Proceedings of Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Biomedical Computer Programs, BMDP, 1975. (ed. W.J. Dixon). Univ. of Calif. Press (Berkeley).

Barsom, G., 1973. Lagoon Performance and the State of Lagoon Technology. EPA Tech. Ser. Rept. R2-73-144, Washington, D.C.

Bartsch, A.F., 1961. Algae as a source of oxygen in waste treatment. J. Wat. Pollut. Control Fed. 33(3):239-248.

Bartsch, A.F. and Allum, M.O., 1957. Biological factors in treatment of raw sewage in artificial ponds. Limnology and Oceanography 2(2):77-84.

Bogan, R.H., 1961. Algae aid in sewage nutrient removals. Water and Sewage Works 108(12):453-458.

Bolitho, V.N. and Dipl, T.P., 1964. Some economic aspects of wastewater purification in South Africa. J. Proc. Int. Sew. Purif., Part 3:258-275.

Bopardikar, M.V., 1969. Microbiology of a waste stabilization pond. Advances in Water Pollution Research. Proceedings of the Fourth International Conference, Prague (S.H. Jenkins, ed.) Pergamon Press (Oxford).

Borchardt, J.A. and Azad, H.S., 1968. Biological extraction of nutrients J. Wat. Pollut. Control Fed. 40(10):1739-1754.

Brockett, O.D., 1976. Microbial reactions in facultative Ponds-I. The anaerobic nature of oxidation pond sediments. Water Res. 10:45.

Caldwell, D.H., 1946. Sewage oxidation ponds-performance, operation & design. Sewage Works J. 18:433.

Caldwell, D.H., et al., 1973. Upgrading Lagoons. EPA Tech. Transfer, Washington, D.C.

Canter, L.W. and England, A.J., Jr., 1970. States design criteria for stabilization ponds. J. Wat. Pollut. Control Fed. 42:1840.

Christie, A.E., 1967. Virus reduction in the oxidation pond. Water and Pollution Control 105(5):45-54.

Clare, H.C., Neel, J.K. and Monday, C.A., 1961. Studies of raw sewage lagoons at Fayette, Missouri, 1958-1959 with a resume of 1957-1958 operations. Proc. Waste Stab. Lag. Sym. Kansas City, Mo. August 1-5, 1960, PHS publication 872.

Canter, L.W., Englande, A.J., Jr., & Mauldin, A.F., Jr., 1969. Loading rates on waste stabilization ponds. J. San. Eng. Div. Proc. Amer. Soc. Civ. Engr., 95, SA6:1117.

Cody, R.M., & Tischer, R.G., 1965. Isolation and frequency of occurrence of Salmonella & Shigella in stabilization ponds. J. Wat. Pollut. Control Fed. 37(10):1399-1403.

Corps of Engineers, 1972. Wastewater Management by Disposal on Land. Special Report 171, U.S. Army, Cold Regions Research and Engineering Laboratory, Hanover, N.H.

Coetzee, O.J., Fourie, N.A., 1965. The efficiency of conventional sewage purification works, stabilization ponds, and maturation ponds with respect to the survival of pathogenic bacteria and indicator organisms. J. Proc. Inst. Sew. Purif., Part 3, 210-215.

Davis, N.E., 1963. Oxidation Ponds. In: Proceedings of Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Dawson, R.N. and Grainge, J.W., 1969. Proposed design criteria for wastewater lagoons in arctic and subarctic regions. J. Wat. Pollut. Control Fed. 41(2):237-246.

Decker, P. E., 1963. Basis of design and uses of oxidation ponds in Missouri. In: Proceedings of Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

de la Espino, E. and Aguirre Martinez, Jr., 1975. Evaluation of waste stabilization pond performance in Mexico. Univ. Tex. Center for Research in Water Resources Sum. Vol 6 (12):275.

Dobos, D., 1975. Electrochemical Data Elsevier Scientific Publishing Co. (New York).

England, B., Leach, R. E., B. Adame, and R. Shiosaki, 1967. Virological assessment of sewage treatment at Santee, Calif. In: Transmission of Viruses by the Water Route, (ed. G. Berg), Interscience Publisher (New York), p. 401.

U.S. Environmental Protection Agency, 1972. In: Proceedings of Conference on Land Disposal of Municipal Effluents and Sludges, EPA 902/9-73-001, Region III, New York, N.Y.

U.S. Environmental Protection Agency Water Program, 1973. Secondary Treatment Information. Federal Register 38, 159, August 17, 1973.

Fisher, C. P., Drynan, W. R. and Van Fleet, G. L., 1968. Waste Stabilization Pond Practices in Canada. In: Advances in Water Quality Improvement (ed. E. F. Gloyna and W. W. Eckenfelder), University of Texas Press (Austin), p. 435.

Fitzgerald, G. P. and Rohlich, G. A., 1958. An evaluation of stabilization pond literature. Sew. and Indust. Wastes 30:1213.

Fleming, J. R., 1963. The sewage lagoon in Tennessee. In: Proceedings of Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Foree, E. G. and McCarty, P. L., 1970. Anaerobic decomposition of algae. Environ. Sci. Technol. 4(11):842.

Franzmathes, J. R., 1970. Bacteria and lagoons. Water and Sew. Works 117:90.

Geldreich, E. E., Clark, H. F. and Huff, C. B., 1964. A study of pollution indicators in waste stabilization ponds. J. Wat. Pollut. Control Fed. 36:1372.

Gaillard, J. R. and Crawford, J., 1964. The performance of algae ponds in Durban. J. Proc. Inst. Sew. Purif. Part 3 pp. 221-229.

Gameson, A.L.H. and Saxon, J. R., 1967. Field studies on the effect of daylight on mortality of coliform bacteria. Water Res. 1(3):279-295.

Gates, F. L., 1929. A study of the bactericidal action of ultra-violet light. J. Gen. Physiol. 13:231-260.

Gloyna, E. F., 1968. Basis for waste stabilization pond designs. In: Advances in Water Quality Improvement, Water Resources Symposium No. 1, University of Texas Press (Austin).

Gloyna, E. F., 1971. Waste Stabilization Ponds. World Health Organization (Geneva).

Golterman, H. L., 1971. Methods for Chemical Analysis of Fresh Waters, I.B.P. Handbook No. 8, Blackwell Scientific Publishers, 1971.

Hermann, E. R. and Gloyna, E. F., 1958. Waste Stabilization Ponds III. Formulation of design equations. Sew. and Indust. Wastes 30(8):963-975.

Hok, J. T., 1963. Die-away of Salmonella abortus equi in oxidation ponds. In: Proceedings of a Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Hodgson, H. T., 1964. Stabilization ponds for a small African urban area. J. Wat. Pollut. Control Fed. 36(1):51-68.

Hopkins, G. J. and Hopkins. O. C., 1963. Waste stabilization lagoons. In: Proceedings of a Symposium on Waste Treatment by Oxidation Ponds, Nagpur Central Public Health Engineering Research Institute.

Horning, W. B., Porges, B., Clarke, H. F. and Cooke, W. B., 1964. Waste Stabilization Pond Study, Lebanon, Ohio. USPHS Publ. 999-WP-16.

Hsu, Y. C. and Kruse, C. W., 1967. Survival of S. typhi in sewage oxidation ponds. J. San. Engr. Div., Amer. Soc. Civ. Engrs. 93(SA1):92.

Johnson, W. K., 1968. Removal of Nitrogen by Biological Treatment. In: Advances in Water Quality Improvement, Water Resources Symposium No. 1, (ed. E. F. Gloyna and W. W. Eckenfelder), The University of Texas (Austin), pp. 178-189.

Joshi, N. R., Parhad, N. M., and Rao, N. U., 1973. Elimination of Salmonella in Stabilization Ponds. Water Res. 7(9): 1357-1365.

Katzenelson, E.B., Feitch, B. and Shuval, H.I., 1976. Spray Irrigation with Wastewater: the Problem of Aerosolization and Dispersion of Enteric Microorganisms, presented at the International Conference on Viruses in Water, Mexico City.

Kharkar, C.B. and Tiwari, A.R., 1963. Sewage Treatment in Stabilization Ponds at Bhilai. In: Proceedings of a Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Klock, J.W., 1971. Survival of coliform bacteria in waste-water treatment lagoons. J. Wat. Pollut. Control Fed. 43(10):2071-2083.

Kott, Y., 1973. Hazards associated with the use of chlorinated oxidation pond effluents for irrigation. Water Res. 7:853-62.

Little, J.A., Carroll, B.J. and Gentry, R.E., 1970. Bacteria removal in oxidation ponds. Proc. 2nd Inter. Sym. Waste Treatment Lagoons, Kansas City, Mo. 141-151.

Loehr, R.C. and Stephenson, R.L., 1965. An oxidation pond as a tertiary treatment device. Proc. San. Engin. Div. Amer. Soc. Civ. Engrs. 91(SA3):31-44.

McGarrey, G. and Bouthillier, H., 1966. Survival of S. typhi in sewage oxidation ponds. J. San. Engin. Div. Amer. Soc. Civ. Engrs. 92(SA4):33-43.

Mackenthum, K.M. and McNabb, D.D., 1961. Stabilization pond studies in Wisconsin. J. Wat. Pollut. Control Fed. 33:1234.

McKinney, R.E., Dornbush, J.N. and Vennes, J.W., 1971. Waste Treatment Lagoons-State of the Art. US EPA, Water Pollution Control Research Series, No. 17090, EHX 07/71.

Malherbe, H.H. and Strickland- Cholmley, M., 1967. Survival of viruses in the presence of algae. Transmission of Viruses by the Water Route. (ed. G. Berg), Interscience Publishers (New York).

Malone, R., Jr. and Bailey, T.L., 1969. Oxidation ponds remove bacteria. Water and Sewage Works 116,4:136.

Marais, G.V.R., 1966. Design, operation, and performance of waste stabilization ponds. Bulletin World Health Organization 34(5):737-763.

Marais, G.V.R., 1974. Faecal bacterial kinetics in stabilization ponds. Environ. Engin. Div. Amer. Soc. Engrs. 100 (EE1):119-139.

Meron, A., Rebhun, M. and Sless, B., 1965. Quality Changes as a function of detention time in wastewater stabilization ponds. J. Wat. Pollut. Control Fed. 37(12):1657-1670.

Merz, R.C., Merrell, J.C., and Stone, R., 1957. Investigation of primary lagoon treatment at Mojave, California. Sew. and Indust. Wastes 29(2):115.

Middlebrooks, E.J., Panagiotou, A.J. and Williford, H.K., 1965. Sludge accumulation in municipal sewage lagoons. Wat. Sewage Works 112:63-68.

Mills, D.A., 1961. Depth and loading rates of oxidation ponds. Water and Sew. Works 108(9):343-346.

Myers, 1949. Studies of Texas Sewage Lagoons. Public Wks. (Dec. 1949).

National Association of State Universities and Land Grant Colleges, 1973. Proceedings of Joint Conference on Recycling Municipal Sludges and Effluents on Land. Washington, D.C.

Neel, J.K. and Hopkins, G.J., 1956. Experimental lagooning of raw sewage Sew. and Indust. Wastes 28(11):1326-1356.

Neel, J.K. McDermott, J.H., and Monday, C.A., Jr., 1961. Experimental lagooning of raw sewage at Fayette, Missouri J. Wat. Pollut. Control Fed. 33:603.

Nemerow, N.L. and Bryson, J.C., 1963. How efficient are oxidation ponds. Wastes Engr. 34:133-135.

Nichols, W.H., 1973. Growth media-freshwater. In: Handbook of Phycological Methods: Culture Methods and Growth Measurements, (ed. J.R. Stein), Cambridge University Press (London).

Nupen, E.M., 1970. Virus studies on the Windloek Wastewater Reclamation Plant (South-West Africa) Water Research 4(10):661-672.

O'Connell, W.J., Jr., and Gray, H.F., 1944. Emergency land disposal of sewage. Sewage Works J. 16(4):729.

Olson, O.O., Van Heuvelen, W. and Vennes, J.W., 1968. Combined industrial and domestic waste treatment in waste stabilization lagoons. J. Wat. Pollut. Control Fed. 40(2):214-222.

Oswald, W.J., 1963. Fundamental factors in stabilization pond design. In: Advances in Biological Waste Treatment (ed. W.W. Eckenfelder and J. McCabe), Pergamon Press (New York), pp. 357-393.

Oswald, W.J., 1968. Advances in anaerobic pond systems design. In: Advances in Water Quality Improvement Water Resources Symposium No. 1, (ed. E.F. Gloyna and W.W. Eckenfelder), University of Texas (Austin), pp. 409-426.

Oswald, W.J. and Gotaas, H.B., 1957. Photosynthesis in sewage treatment. Transactions Amer. Soc. Civ. Engin. 122:73-97.

Oswald, W.J., Gotaas, H.B., Golueke, C.G., and Kellen, W.R., 1957. Algae in waste treatment. Sew. and Industs. Wastes. 29(4):437.

Parhad, N.M. and Rao, N.U., 1963. Preliminary studies on the microbiology of oxidation ponds. In: Proceedings of a Symposium on Waste-Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Parhad, N.M., and Rao, N.U., 1974. Effect of pH on survival of Escherichia coli. J. Wat. Pollut. Control Fed. 46(5): 980-986.

Parker, C.D., 1962. Microbiological aspects of lagoon treatment. J. Wat. Pollut. Control Fed. 34:149.

Parker, C.D., Bull, G., Beechey, M. and R.R. Bayliss, 1963. The Anaerobic lagoon. In: Proceedings of Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Parker, C.D., Jones, H.L., and Greene, N.C., 1959. Performance of large sewage lagoons at Melbourne, Australia. Sewage and Indust. Wastes 31:133.

Parker, C.D., Jones, H.L., and Taylor, W.S., 1950. Purification of sewage in lagoons. Sewage Ind. Wastes 22:760.

Porges, R., 1963. Industrial waste stabilization ponds in the United States. J. Wat. Pollut. Control Fed. 35(4): 456-468.

Pound, C.E. and Crites, R.W., 1973. Wastewater Treatment and Reuse by Land Application, Col. III, EPA-66012-73-0066, US Environmental Protection Agency, Washington, D.C.

Pratt, R., et al., 1944. Chlorellin, and antibacterial substance from Chlorella Science 99:351.

Ridenour, G. M., 1933. Studies of the effect of a small impounding reservoir on stream purification. Sew. and Indust. Wastes 5(2):319.

Shindala, A. and Mahloch, J. L., 1974. Evaluation of three waste stabilization ponds in series. Water and Sewage Works 121(3):57-59.

Shuval, H. I., 1969. Detection and control of enteroviruses in the water environment. In: Development in Water Quality Research, Proceedings of the Jerusalem International Conference on Water Quality and Pollution Research, Ann Arbor-Humphrey Science Publishers (London).

Sidio, A. D., Hartman, R. T. and Fugazzotto, P., 1961. First domestic waste stabilization pond in Pennsylvania. Pub. Health Reports 76:201.

Simmonds, M. A., 1973. Experience with algal blooms and removal of phosphorus from sewage. Water Res. 7:255-264.

Slanetz, L. W., Bartley, C. H., Metcalf, T. G. and Nesman, R., 1972. Survival of Enteric Bacteria and Viruses in Oxidation Pond Systems. Research Report No. 6, Water Resources Research Center, University of New Hampshire (Durham).

Sobsey, M. D. and Cooper, 1973. Laboratory studies on the survival of poliovirus in algal-bacterial wastewater treatment plants. In: Proc. Thirteenth Water Quality Conference, Virus and Water Quality Occurrence and Control. University of Illinois (Urbana-Champaign).

Sobsey, M. D. and Cooper, R. C., 1973. Enteric virus survival in algal-bacterial water treatment systems-I. Laboratory Studies. Water Res. 7:669-685.

Sorber, C. A., Bausum, H. T., Schaub, S. A. and M. J. Small, 1976. A Study of Bacterial Aerosols at a Wastewater Irrigation Site, presented at 48th Annual Conference of the Water Pollution Control Federation, Miami Beach.

Sorber, C. A., Schaub, S. A., Bausum, H. T., 1974. An Assessment of a Potential Virus Hazard Associated with Spray Irrigation of Domestic Wastewaters. In: Virus Survival in Water and Wastewater Systems, Water Resources Symposium No. 7 (ed. J. F. Malina and B. P. Sagik), Center for Research in Water Resources, The University of Texas (Austin).

Sorber, C. A., Schaub, S. A., and Guter, K. J., 1972. Problem Definition Study: Evaluation of Health and Hygiene Aspects of Land Disposal of Wastewater at Military Installations: USAMEERU Report No. 73-02, AD No. 752122, US Army Medical Environmental Engineering Research Unit, Edgewood Arsenal, Md.

Sproul, O. J., 1974. Removal of Viruses by Treatment Processes, presented at the International Conference on Viruses in Water, Mexico City.

Stahl, J. B. and May, D. S., 1967. Microstratification in waste treatment ponds. J. Wat. Pollut. Control Fed. 39(1):73-88.

Stouse, D. C., 1964. Waste stabilization ponds in Iowa-a survey. Water Works and Wastes Engineering 1(8):52.

Stratton, F. E., 1969. Nitrogen losses from alkaline water impoundments. J. Sanit. Engng. Div. Amer. Soc. Civ. Engrs. (SA2):223-231.

Stumm, W. and Morgan, J. J., 1962. Stream Pollution by Algal-Nutrients. Harvard University Sanitary Engineering Reprint No. 45-Reprinted from the Transactions of the 12th Annual Conference on Sanitary Engineering, University of Kansas pp. 16-26.

Suwannakarn, V., 1963. Temperature Effects on Waste Stabilization Pond Treatment. Ph.D. thesis, University of Texas, Austin.

Suwannakarn, V. and Gloyna, E. F., 1963. Temperature effects on waste stabilization pond treatment. In: Proceedings of a Symposium on Waste Treatment by Oxidation Ponds, Nagpur, Central Public Health Engineering Research Institute.

Svore, J. H., 1968. Waste stabilization pond practices in the United States. In: Advances in Water Quality Improvement, (ed. E. F. Gloyna and W. W. Eckenfelder). University of Texas Press (Austin).

Towne, W. W., Bartsch, A. F. and Davis, W. H., 1957. Raw sewage stabilization ponds in the Dakotas. Sew. and Ind. Wastes 29:377.

Ullrich. A. H., 1967. Use of wastewater stabilization ponds in 2 different systems. J. Wat. Pollut. Control Fed. 39:965.

Van Eck, H., 1959. Sewage stabilization ponds: a critical review. J. and Proc. Insti. Sew. Purif., Part 3:320-334.

Van Heuvelen, W., Smith, J. K. and Hopkins, G. F., 1960. Waste stabilization lagoons-design, construction, and operation practices among Missouri Basin States. J. Wat. Pollut. Control Fed. 32(9):909.

Van Heuvelen, W., and Svore, J.H., 1954. Sewage lagoons in North Dakota. Sew. and Indust. Wastes 26,6:771.

Wachs, A.M. and Berend, A., 1968. Extradeep ponds. In: Advances in Water Quality Improvement, Water Resources Symposium No.1 . (ed. E.F. Gloyna and W.W. Eckenfelder), The University of Texas (Austin), pp. 450-456.

Warrington, S.L., 1952. Effects of using lagooned sewage effluent on farmland. Sew. and Indust. Wastes 24:1243.

Williford, H.K. and Middlebrooks, E.J., 1967. Performance of field scale wastewater treatment lagoons. J. Wat. Pollut. Control Fed. 39(12):2008-2019.

Young, J.H., Jr., 1974. Facultative Waste Stabilization Pond Processes, Performance, Design, and Predictive Models. MSc Thesis, University of Texas, Austin.

Yousef, I., 1962. Coliforms in waste stabilization ponds. MSc Thesis, University of Texas, Austin.

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